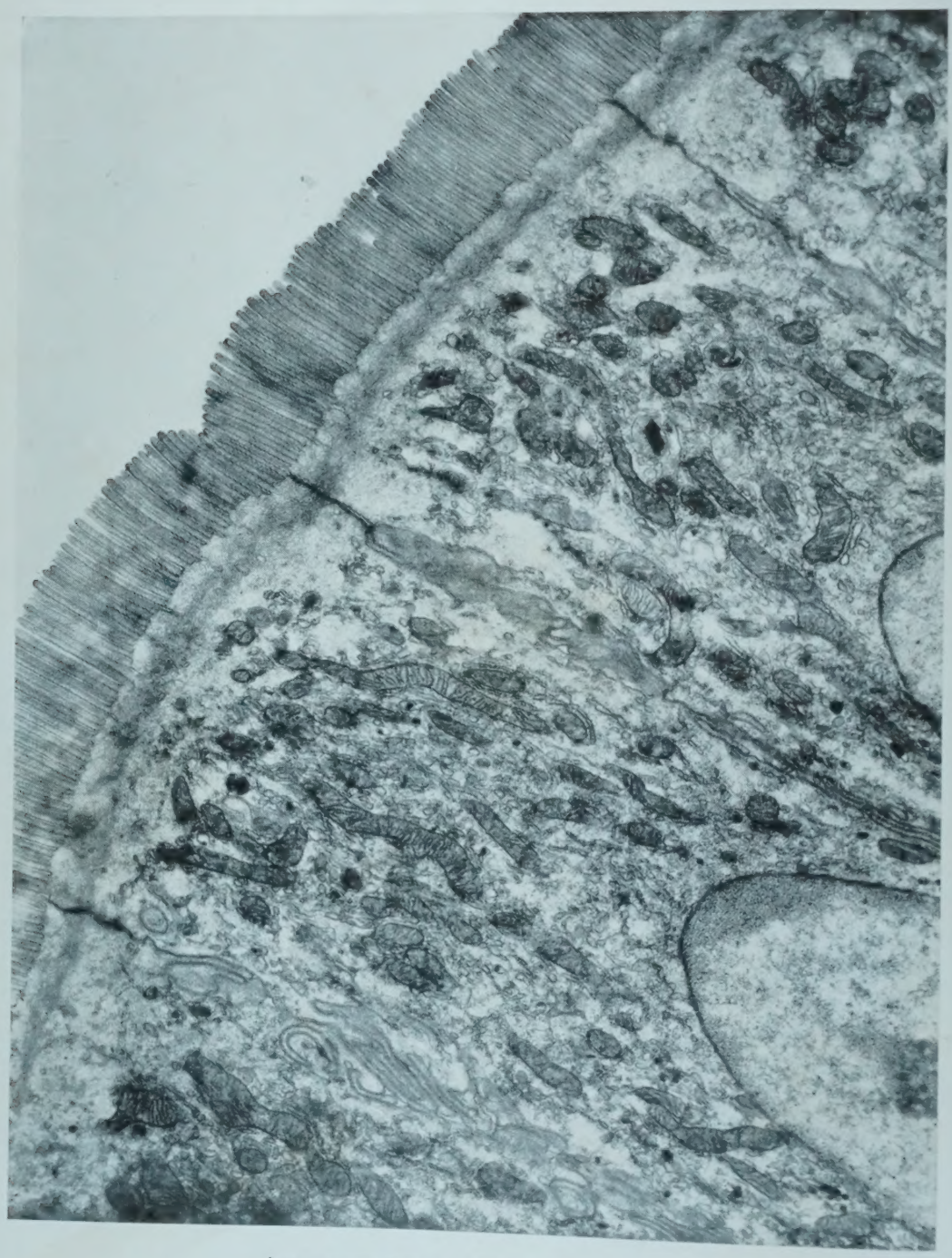

Absorptive Cells of Hamster Jejunum.
Kindly provided by Dr. Elliott W. Strauss.

INTESTINAL ABSORPTION

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THIS BOOK IS DEDICATED TO

MERKEL HENRY JACOBS

who first introduced me to the problems
of cell permeability and who has been
a source of inspiration ever since

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T. Hastings Wilson

Contents

CHAPTER 1

Introduction

MORPHOLOGICAL FEATURES OF THE SMALL INTESTINE

There are a number of unique morphological features in the small intestine. An important aspect of the structure of this organ is the special means of enormously increasing the epithelial surface area for absorption. Figure 1 shows the three different modifications of a simple cylinder which permit just such an increase of surface area. It is estimated that the surface area may be increased six hundred fold by the unique morphological modifications of the digestive tube.

The folds of Kerkring or *valvulae conniventes* are folds of mucosa found in the human small intestine. The folds are most prominent in the duodenum and upper jejunum but continue into the ileum.

The villi of the small intestine are long, finger-like processes from 0.5 to 1.5 mm. in length which project into the lumen. Figure 2 shows the appearance of the villi in a specimen of normal human jejunum obtained by biopsy. The villi are lined with two cell types: the columnar absorbing cell and the goblet, or mucus-secreting cell. Between the bases of the villi are glandlike structures about 400 μ long called the crypts of Lieberkühn. As will be indicated later, the crypt region is largely generative in function. There are, however, secretory cells, the argentaffine cells and Paneth cells, which produce and probably secrete some products the identity of which is still largely unknown. Villi con-

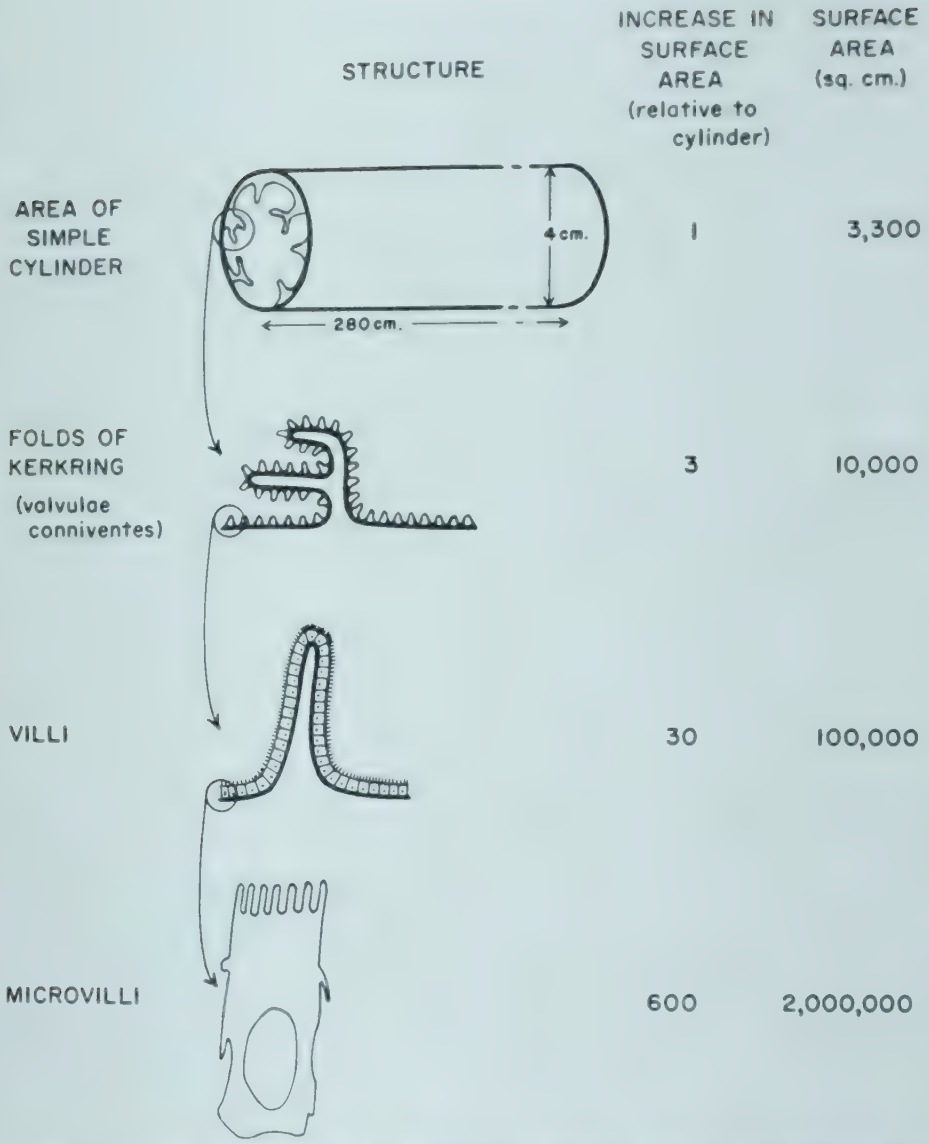

Figure 1. Three mechanisms for increasing surface area of the small intestine. Length of human small intestine obtained from Hirsch et al.,[28] radius from Gray.[24] The increase in surface area due to folds of Kerkring is an estimate of the author. Increase in area due to villi estimated as 8 in rat and pigeon by Verzár.[55] Increase in area due to microvilli in the mouse was estimated as 14 by Zetterqvist[59] and in the rat as 24 by Palay and Karlin.[48]

tain strands of smooth muscle, parallel with the long axis. These muscle strands are probably responsible for the pumping action of the villi, which is important in absorption of substances by the lymphatic route (see the discussion by Verzár[55]).

An outline of the ultra-structure of the columnar absorbing cell is given in Figure 3. The luminal border of the cell is lined with finger like projections (microvilli) about 1 μ long and 0.1 μ wide. Zetterqvist[59] found about 600 microvilli per cell and 50,000,000 per

Figure 2. Villi of human small intestine. Biopsy specimen taken from a normal adult. X 12.5. (Rubin et al.: Gastroenterology, *38*, 1960.)

square millimeter of intestinal surface in the mouse. A high magnification of these structures in the rat is given in Figure 4. Below the microvilli is an area called the terminal web, containing a mesh of thin filaments. The apical cytoplasm of the cell contains considerable numbers of rod-shaped mitochondria oriented parallel to the long axis of the cell. The Golgi apparatus is quite well-developed, especially in the cells of the crypts. Two types of endoplasmic reticulum are found in these cells: agranular reticulum (smooth surface E.R.) and granular reticulum (membranes studded with ribonucleoprotein particles). Agranular reticulum is seen mainly in the apical cytoplasm, usually as small vesicles 30 mμ in diameter.[48] In the supranuclear region of the cell is granular reticulum, which accounts for the basophilia seen with light microscopy. The endoplasmic reticulum plays a prominent role in fat absorption and triglyceride synthesis, as will be discussed in Chapter 7.

Blood capillaries in the lamina propria consist of a single layer of endothelial cells with a basement membrane below. Cells overlap at their margins with adjacent cells, as in other tissues. The wall itself is perforated with fenestrae 20 to 50 mμ wide.[5] As with other capillaries, small pinocytotic vesicles are present in the cytoplasm of the cells.

Lymphatic capillaries can be distinguished from blood vessels by a number of characteristics. The minimal thickness of the lymphatic is five to six times that of a blood capillary. Unlike blood vessels there are no fenestrations in the walls of the lymphatic capillary. Furthermore, the lymphatic capillary lacks a basement membrane.[20] Palay and Karlin state that the endothelial cells of the lacteal are occasionally disjointed. "The resulting interruption in the coherence of the wall leaves a passage of variable dimensions through which interstitial fluid and lymph may communicate."[48]

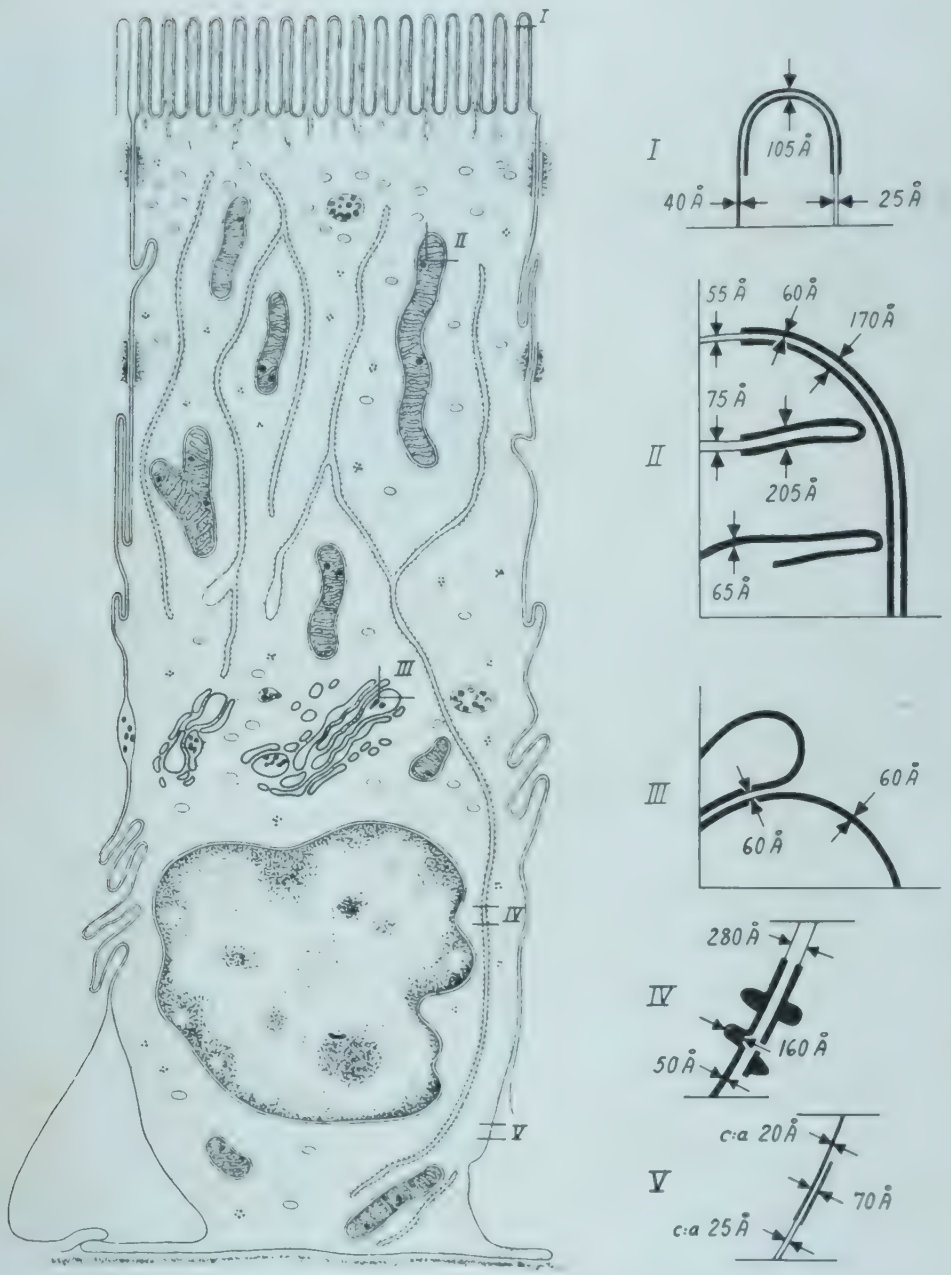

Figure 3. Schematic drawing of columnar absorbing cell. To the right the dimensions are given for *I.* plasma membrane on the free cell surface. *II.* mitochondrial membranes, *III.* γ-cytomembranes, *IV.* α-cytomembranes and *V.* plasma membrane on the cell surface towards the space between the bases of the cells. (Zetterqvist: Karolinska Institutet, Aktiebolaget Godvil.)

ROUTES OF ABSORPTION (Blood or lymph)

Both the lymphatic and blood vascular systems are extremely well developed in the small intestine. Each villus is provided with a rich network of small vessels of both types. In 1887 Mall[41] made a careful

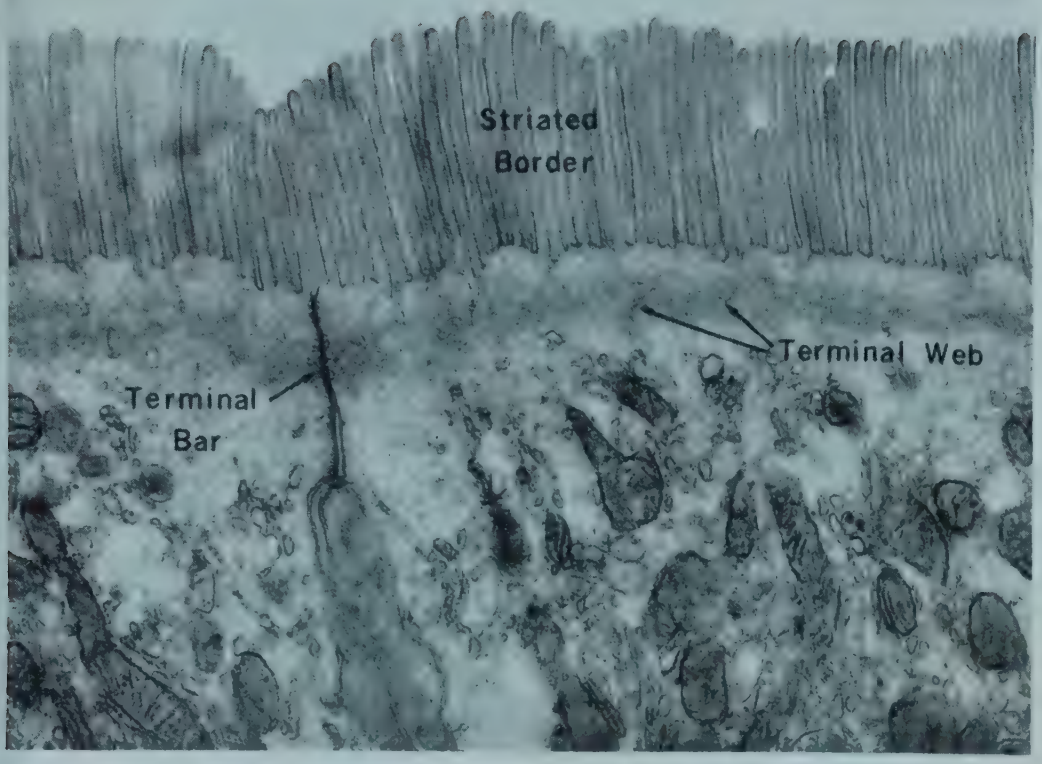

Figure 4. Microvilli of the absorptive cell of hamster small intestine. Picture taken by Dr. Elliott W. Strauss. (Bloom and Fawcett: Histology, 8th ed.)

study of both lymphatic and blood vessels of the intestine. Figures 5 and 6 are black-and-white reproductions of two of the original color plates from Mall's paper.

Lymphatics

The first observations on the lymphatic route of absorption were apparently made in 1627 by Asellius[3] who noted the milkiness of the intestinal lymphatics of a dog following a fat meal. More recent studies indicate that virtually all absorbed triglycerides containing long-chain fatty acids appear in the lymph. Bloom et al.[9] were able to collect 70 to 92 per cent of absorbed radioactivity in the lymph when C^{14}-labeled palmitic acid (in triglyceride) was fed to rats. In another series of experiments, the recovery was 81 to 95 per cent in the lymph.[10] Chaikoff et al.[15] showed that 94 to 100 per cent of absorbed cholesterol appeared in the thoracic duct lymph in the rat.

Another substance that is probably absorbed exclusively by the lymphatics is protein. Alexander, Shirley, and Allen[1] fed egg albumin to adult dogs and measured unaltered albumin by immunological methods in the thoracic duct, portal blood, and systemic blood. They found that egg albumin appeared in the lymph before it appeared in the systemic blood and never was detected in the portal blood. After feeding

Figure 5. Blood vessels of the intestinal mucosa of the dog. (Mall: Abh. Sächs Ges. Wiss., *14*, 1887.)

botulinum toxin to rabbits. May and Whaler[42] were able to recover most of it in the lymph. In addition, continuous drainage of the thoracic duct provided partial protection from the toxemia following oral administration of the toxin.

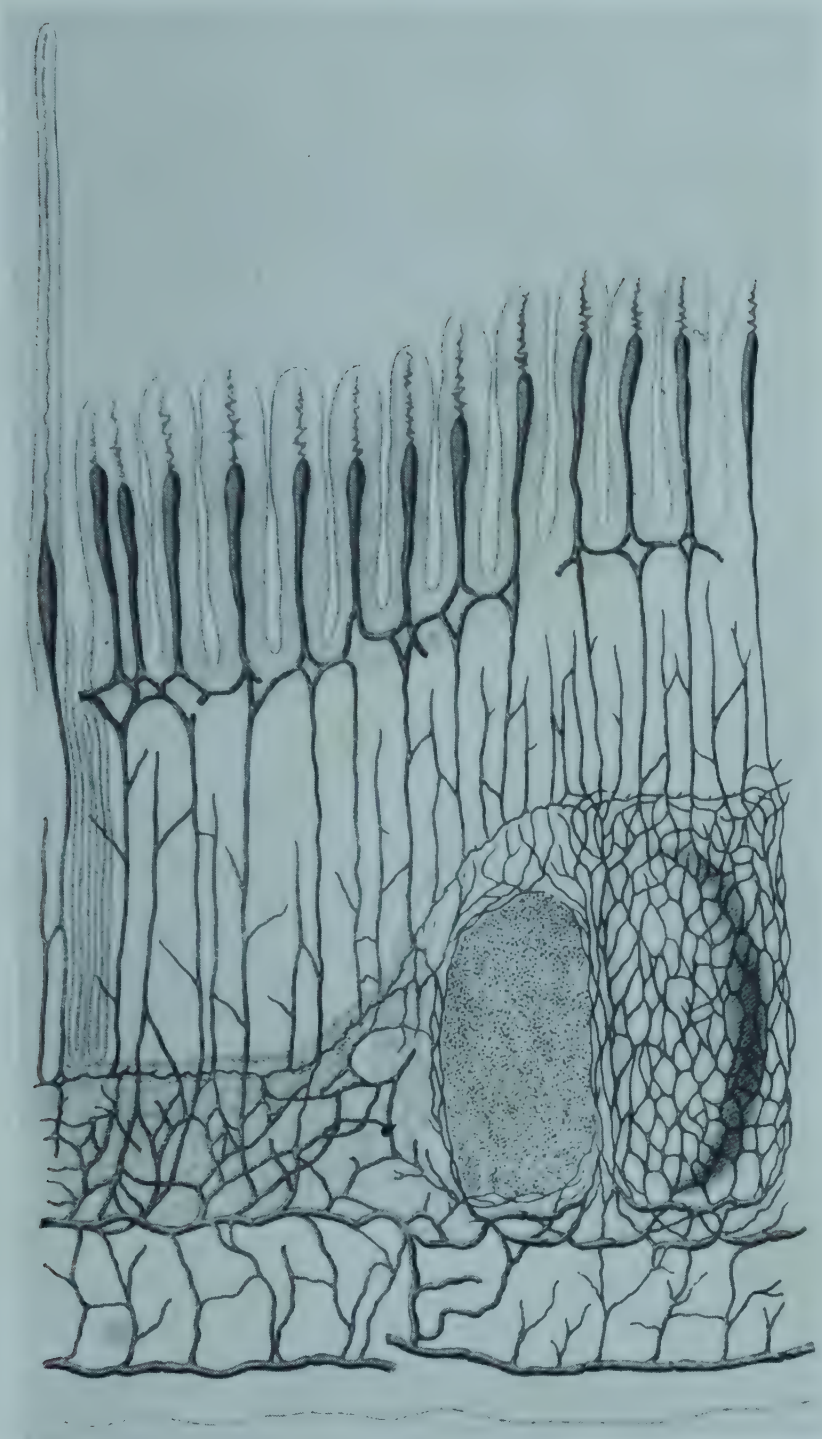

Figure 6. Lymphatic vessels of the intestinal mucosa of the dog (Mall: Abh. Sächs. Ges. Wiss., *14*, 1887.)

Intestinal absorption of unaltered colostrum proteins occurs in the newborn of a number of species of animals (see Chapter 10). The route of this absorption was studied by Comline, Roberts, and Titchen.[16] They found that in the newborn calf, gamma globulins of the ingested

colostrum were absorbed into the lymphatics and not into portal blood.

Why do absorbed proteins, triglycerides, and cholesterol find their way exclusively into the lymphatic vessels? A working hypothesis has been suggested by Landis.[38] According to this hypothesis substances of large molecular weight (e.g., protein molecules and lipid particles), following extrusion from the epithelial cell, cross the basement membrane into the connective tissue spaces. These substances cannot enter the blood capillaries at any appreciable rate because of the permeability barrier of the capillary basement membrane. Entrance into the lymphatic capillary, on the other hand, is readily accomplished because of the lack of a basement membrane and the frequent separations of endothelial cells resulting from the continuous movement of the tissue from peristalsis and the pumping action of the villi. Once within the lacteals, movement is induced by the slight local hydrostatic pressure gradients resulting from rhythmic motion of the surrounding tissue.

This hypothesis is based upon inferences derived from both morphological and physiological data. While the capillary endothelium of the intestine has fenestrae large enough to admit many large molecules, the basement membrane must effectively exclude them since proteins appear almost exclusively in the lymphatics. On the other hand, the endothelial cells of the lymphatic capillaries must have somewhat larger spaces between them than are normally seen in osmium-fixed tissue, as large particles do in fact enter lymphatics with considerable ease. The many substances known to enter lymphatic capillaries (including nucleated erythrocytes[19]) are discussed in detail elsewhere.[17, 18, 52, 57]

Some confusion exists in the literature concerning the role of lymphatics in fat absorption because ligation of the thoracic duct has little effect on lipid absorption. Furthermore, the thoracic duct is not essential for life. The explanation for these apparently anomalous facts was established some years ago by Lee[39] and others[2, 5, 23] who showed that ligation of the thoracic duct results in collateral circulation either to the right lymphatic duct (which drains into the right subclavian vein) or to the lymphatico-venous connections between the thoracic duct and the azygos vein. When the thoracic duct is intact these collaterals are apparently very small and physiologically unimportant.

Blood Capillaries

Both the blood capillaries and lymphatic vessels are freely permeable to all small molecular weight compounds and lipid-soluble compounds of moderate size. For such compounds the blood capillaries constitute the major route of absorption as a result of rapid blood flow. This idea was clearly stated by Hendrix and Sweet in 1917[25]: "It is suggested that the practically complete absorption of protein and carbohydrate by

the blood is not due to a selective resorption, but to the almost infinitely large volume of blood, as compared to the volume of lymph, which flows through the walls of the intestine."

Data are available for a quantitative comparison between the flow rates of blood and lymph in a variety of animals (Table 1). The flow rates on the basis of body weight are remarkably similar in the rat, dog and human. Bollman, Cain, and Grindlay[11] found that about 80 per cent of the thoracic duct lymph of the rat was derived from the intestinal tract and about 20 per cent from the liver. The flow rates for the thoracic duct, therefore, give a reasonable estimate of intestinal lymph flow under most conditions. Blood flow in each case is approximately 500 to 1,000 times that of lymph. If such compounds as glucose and amino acids entered both types of vessels with equal ease over 99 per cent would be absorbed through the portal vein.

Table 1. COMPARISON BETWEEN FLOW RATE OF BLOOD AND LYMPH

ANIMAL	FLOW RATE (ML./HR./KG.)		RATIO
	PORTAL VEIN	THORACIC DUCT	BLOOD/LYMPH
Rat (fed)	2,250	4.4	500
(fasted)	1,700	2.6	650
Dog	1,280	2	600
Man	1,200	1-2	600-1,200

For the rat: portal vein data taken from Reininger and Sapirstein,[50] the thoracic duct data from Reinhardt and Bloom,[49] and Bollman et al.[11] For the dog: portal vein data from Blalock and Mason[7] and thoracic duct data averaged from ten authors summarized by Yoffey and Courtice.[57] For man: portal vein flow taken as 80 per cent of the hepatic blood flow as measured by Bradley et al.[13] and thoracic duct data summarized by Yoffey and Courtice.[57]

Effect of Feeding on Flow Rates of Blood and Lymph

It has long been thought that during meals there is a redistribution of blood from various parts of the body to the intestines. The experimental evidence in the rat,[50] dog[27] and man[14] indicates that there is only about a 30 per cent increase in blood flow through the splanchnic area following a meal. Reininger and Sapirstein[50] have shown that in the rat the blood flow to many regions of the body increases following a meal and that the increase in the splanchnic area is no greater than that found in the other regions of the body.

There is, however, a distinct increase in lymph flow after a meal. Yoffey and Courtice[57] have reviewed the factors that increase lymph flow in different animals. Lipids and some solutions, especially 1 per cent NaCl solutions, greatly stimulate lymph production. It should be noted, however, that even with a five- to tenfold increase in lymph flow, the rate of portal blood flow will still be two orders of magnitude greater.

SOME QUANTITATIVE ASPECTS OF INTESTINAL ABSORPTION

It seems appropriate in an introductory chapter to point out the enormous differences in the rate of absorption of different substances. Table 2 shows the estimated capacity of the human intestine to absorb a series of substances. In those cases in which the capacity of the intestine greatly exceeds the normal demand placed upon it, a number of assumptions have been made in the calculation. It is clear that there are prodigious differences in the rate of absorption even if there is an error of as much as an order of magnitude in a few of the estimates. The capacity of the intestine to absorb water molecules is approximately 12 orders of magnitude greater than its capacity to absorb vitamin B_{12}. The absorption rate of this vitamin in an untreated case of pernicious anemia is probably about 1/100 that given in the table for normal individuals. From this it is clear that such terms as "poorly absorbed" and "well absorbed" are meaningless without some quantitative qualification.

Table 2. COMPARISON OF TRANSPORT CAPACITY FOR DIFFERENT NUTRIENTS

SUBSTANCE	ABSORPTIVE CAPACITY IN MAN PER DAY	
	GR.	mM.
Water	18,000	1,000,000
Glucose	3,600	20,000
Amino acids	600	5,000
Triglycerides	700	900
Cholesterol	4	10
Iron	0.012	0.2
Vitamin B_{12}	0.000001	0.000001

Water: Borgström et al.[12] found 500 ml. test meal diluted to 1,500 to 2,500 in duodenum and mostly resorbed at the level of the midgut. This amounted to absorption of about 1,500 ml. in 4 hours in one half of the intestine. This value was multiplied by 12 to obtain the value in the table. Glucose: 250 mg. glucose fed to human subjects[26] is probably completely absorbed in three to four hours and probably only in the first half of the gut. Amino acids: Borgström et al.[12] found 25 gm. protein completely absorbed in upper quarter of gut in four hours. Average mole weight taken as about 120. Triglycerides: 30 gm. corn oil absorbed in upper quarter of gut in four hours.[12] Iron: of 100 mg. dose, 12 mg. absorbed.[54] Vitamin B_{12}: B_{12} absorbing mechanism is saturated about 1.5 μg.[22] It should be emphasized that these calculations are extremely rough approximations because of the many assumptions which must be made.

There are also wide differences in the efficiency with which the intestine absorbs the substances ingested in the diet. Under normal conditions the usual quantities of ingested water, glucose, amino acids, and triglyceride are 95 to 100 per cent absorbed. On the other hand, most diets contain more di- and trivalent anions and cations than can be absorbed by the intestine.

TURNOVER OF INTESTINAL EPITHELIUM

It was once thought that the large number of mitoses seen in the crypts of Lieberkühn represented the production of new cells necessary to replace the damaged cells of the villi injured mechanically by the movement of food down the intestinal tract. Leblond and Stevens[36] reinvestigated this question in 1948 and found that mitotic activity was the same with or without food in the lumen of the intestine and persisted for at least five days of complete fasting. They proposed that new cell formation was not a repair phenomenon but a normal physiological process of cell renewal, coupled with cell extrusion.

An important experiment was performed by Friedman,[21] who found that goblet cells in the crypts, swollen with low doses of x-rays, migrated up the sides of the villi. Leblond, Stevens, and Bogoroch[37] injected a single dose of radioactive inorganic phosphate into animals and found that the nuclei of the dividing cells of the crypts became radioactive in two hours. As the blood level of radioactive phosphate quickly fell to low levels the subsequent groups of dividing cells in the crypts were nonradioactive. The group of cells labeled immediately after the injection could then be followed by autoradiographic methods. After 18 to 24 hours these labeled cells were found on the sides of the villi, at 36

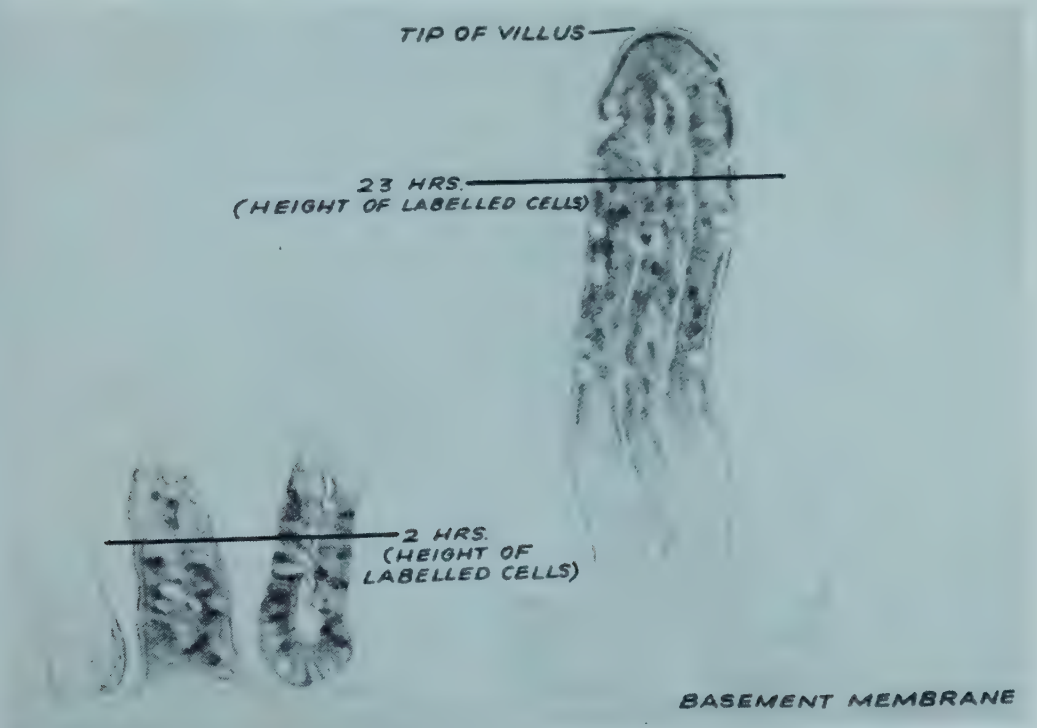

Figure 7. Diagram of autoradiograph of intestinal mucosa of a rat following a single injection of labeled thymidine. Radioactivity is confined to the crypt region 2 hours after the injection but approaches the tip of the villus after 23 hours. (Loran and Althausen: J. Biophys. & Biochem. Cytol., 7, 1960.)

hours at the tips of the villi, and later lost into the lumen. These observations have been repeated with a variety of labeled compounds (see Leblond and Walker[38] for a review). The use of tritiated thymidine has become the method of choice as it specifically labels the DNA of the nuclei and is especially suitable for good resolution on autoradiographs. Figure 7 shows the general method employed. The turnover time for the epithelium of the rat, cat, mouse, and man is given in Table 3.

Table 3. TURNOVER TIME* OF INTESTINAL EPITHELIUM

AREA	SPECIES	TURNOVER TIME (DAYS)	AUTHORS
Duodenum	Rat	1.6	Leblond and Stevens[36]
	Cat	2.3	McMinn[43]
	Man	1.8	Bertalanffy and Nagy[6]
Jejunum	Rat	1.4	Widner, Storer, and Lushbaugh[56]
	Mouse	1.8	Knowlton and Widner[32]
Ileum	Rat	1.4	Leblond and Stevens[36]
	Cat	2.8	McMinn[43]

* The time taken for the replacement of the number of cells equal to that in the total population.

Further support for the cell turnover hypothesis comes from a study of Hooper.[29] Isolated closed loops of rat ileum were found to fill rapidly with desquamated epithelial cells and mucus. Since no food passed through the loop, the layer of mucus and cells was undisturbed. After two days a column of desquamated epithelial cells was found immediately above each villus. The cells nearest the tip of the villus looked most nearly like intact cells, while cells further into the lumen were more autolyzed. The histological appearance of the "extrusion zone" and the appearance of extruded cells above the villi strongly suggest that cells are lost from the epithelium at the tips of the villi.

Desquamated epithelial cells in the lumen of the intestine are responsible for many of the enzymes found free in the intestinal tract. Alkaline phosphatase, invertase, lactase (β-galactosidase), peptidase, and other enzymes in the lumen are undoubtedly derived from cell debris. What was once dignified by the name *succus entericus* is probably no more than a mixture of mucus, desquamated epithelium, and a small amount of fluid. Large volumes of intestinal fluid can be obtained from Thiry-Vella loops only in response to mechanical stimulation. Leblond and Walker[38] have calculated that if the data on cell renewal obtained from the rat were applicable to man (which is probably the case[6]), the mass of cells released daily from the entire gastrointestinal tract would weigh about 250 gm. This would make a significant contribution to the

absorptive load, as these cells must be digested and the split products absorbed in the same manner as ingested food.

Apparently the rate of cell proliferation in the crypts and the rate of extrusion can be varied independently.[34, 38] Thus, starvation appears to increase extrusion while x-irradiation or colchicine leads to a reduction in proliferation. Intestinal resection results in increased turnover.[40] Some of these factors are discussed in recent reviews.[34, 58]

MATURATION OF EPITHELIAL CELLS OF THE SMALL INTESTINE

Since the time sequence for the progression of new cells from the crypts up the sides of the villi is known, a rough estimate of the age of any given cell can be obtained. When one tests the enzymatic or transport machinery of epithelial cells a gradation in activity along the villus is usually obtained. Some activities are greatest in the cells of the crypt while others are more developed at the tips of the villi.

It might be expected that the rapidly dividing cells in the crypts

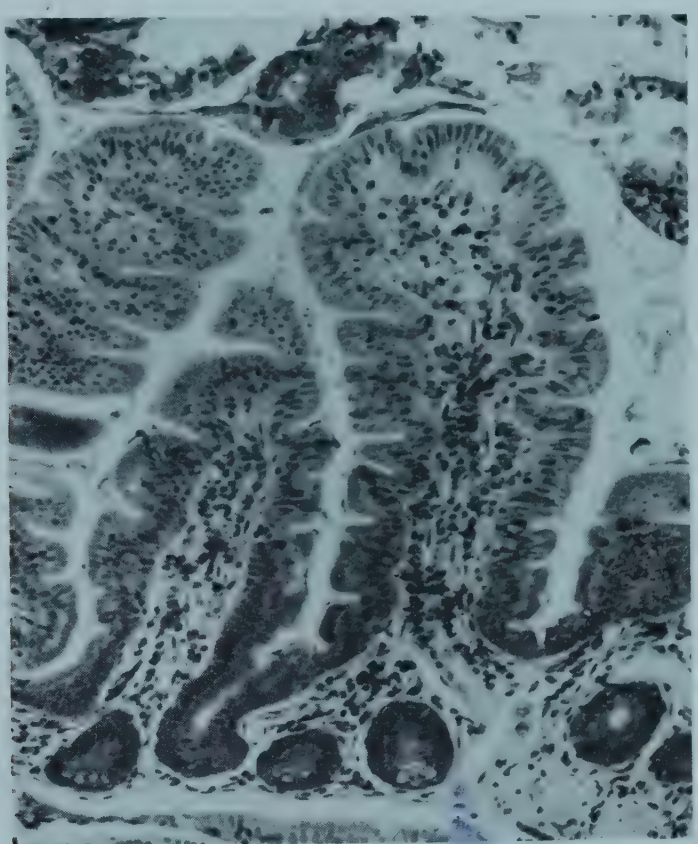

Figure 8. Distribution of cytoplasmic basophilia. Section stained with eosin-methylene blue. X 200. The epithelial lining of the crypts shows a pronounced cytoplasmic basophilia which gradually diminishes as the cells migrate toward the tip of the villus. (Padykula et al.: Gastroenterology, *40*, 1961.)

possess considerable protein synthetic activity. Padykula et al.[47] have shown (Figure 8) that the basophilia of cells in the crypts is much more prominent than that of the villi. Their basophilia is associated with endoplasmic reticulum, which plays an integral part in protein synthesis. S^{35}-labeled methionine, when injected into an animal, rapidly appears in the cells of the crypts,[4, 45] with only a little being found on the villi.

Most of the hydrolytic enzymes associated with the unique digestive function of intestinal epithelium develop during the cells' migration up the villi, for many are absent in the crypts. Figure 9 shows the distribution of esterase in the villi of normal human jejunal mucosa. There appears to be a rather sharp line of demarcation between the villi and crypts, with the latter showing virtually no activity. This distribution is also seen with alkaline phosphatase,[30] leucine amino peptidase[45] and ATPase.[47] It might be anticipated that succinic dehydrogenase, an enzyme associated with mitochondria, might be more active in the cells of the villi than those in the crypts[46, 47] (Figure 10). The histochemistry of other enzymes of the gastrointestinal mucosa has recently been reviewed by Shnitka.[53]

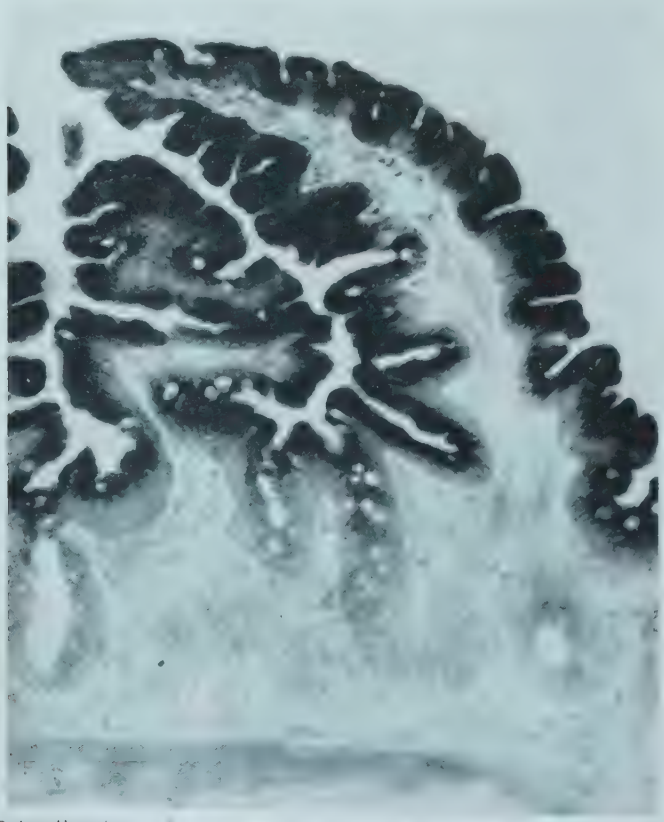

Figure 9. Distribution of esterase in human jejunal mucosa. Very strong esterase activity on sides and tips of villi. Note the fairly abrupt increase in enzyme activity as the cells pass from crypt to villus. X 200. (Padykula et al.: Gastroenterology, *40*, 1961.)

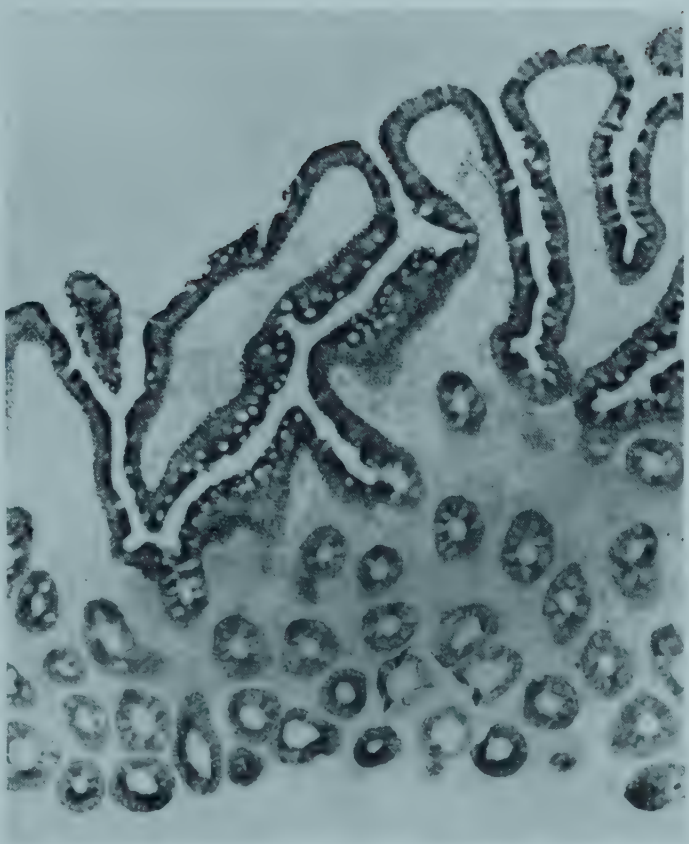

Figure 10. Distribution of succinic dehydrogenase in human jejunal mucosa. Strong activity is found on the villi and in a few cells at the base of the crypts (probably Paneth and argentaffine cells). X 100. (Padykula et al.: Gastroenterology, *40*, 1961.)

Kinter[31] has found that when everted sacs of hamster intestine were incubated with radioactive sugars and amino acids for a minute or less, the epithelial cells at the tips of the villi accumulated these compounds to a considerable degree, while little accumulation was observed in cells at the base of the villi (Figure 11). The unequal distribution does not appear to be the result of poor contact between the medium and the cells at the base of the villi, as similar pictures were obtained with much longer incubation periods (10 to 30 minutes). It was inferred from such studies that the transport capacity of the intestinal epithelium at the tips of the villi is greater than that further down their sides.

There is histochemical evidence that some of the hydrolytic enzymes, such as alkaline phosphatase, are localized at the brush border of the epithelial cell. Johnson and Kugler[30] presented histochemical evidence of a bilaminar distribution of this enzyme, the most prominent band being in the area of the microvilli. A recent observation on the kidney tubule, which also contains this enzyme, may be relevant. Mölbert et al.[44] have developed a method for demonstrating phosphate activity with the electron microscope and found an equal amount of activity

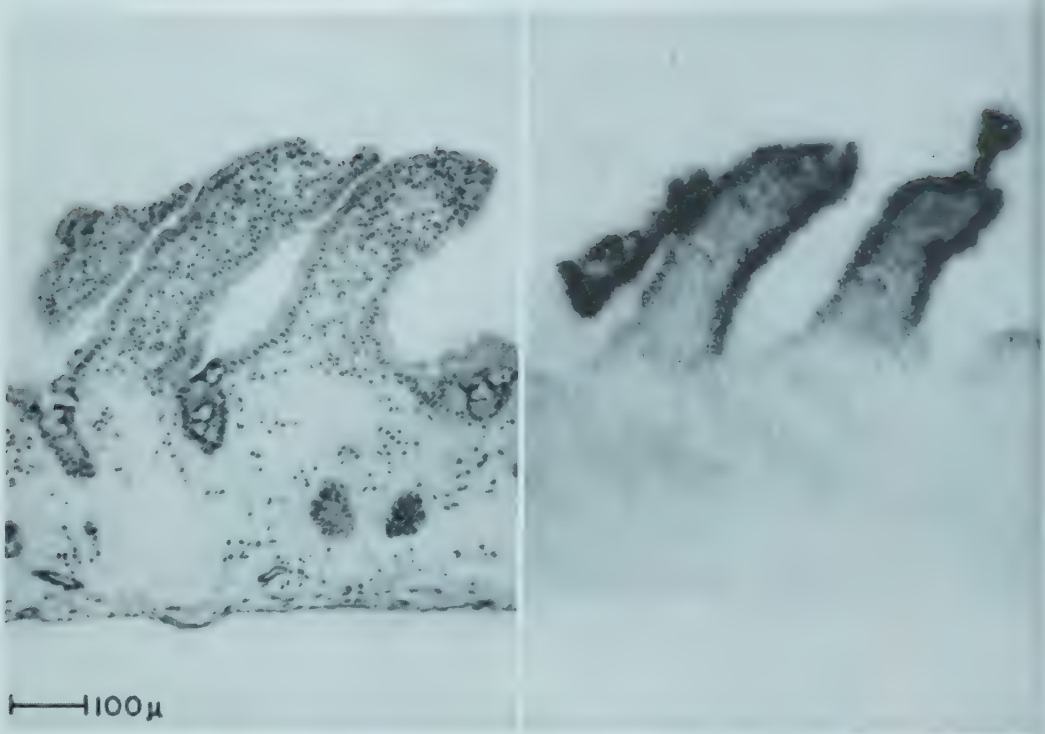

Figure 11. **Distribution of methionine transport activity in hamster jejunum.** Everted sac of hamster jejunum incubated 30 seconds in the presence of C^{14}-labeled methionine, frozen in isopentane at −160° C. and sectioned in a cryostat. Alternate sections were stained with hematoxylin-eosin (left) and exposed to photographic film (right). (Kinter: in Proc. 12th Ann. Conf. Nephrotic Syndrome.)

along the entire cell membrane – microvilli, lateral margins, and basal infoldings. If this were also true of the intestinal epithelium, the high concentration of the enzyme in the brush border would be due to the great concentration of cell membrane (due to microvilli) in this region of the cell. A similar localization of transport activity in the microvilli has been inferred from autoradiographic studies of sugar and amino acid transport.[31] If these inferences are correct, the plasma membrane of the absorptive cell must be a dynamic functional unit of the cell containing a wide spectrum of hydrolytic enzymes plus the machinery for many specialized transport processes.

The absorptive cells in the upper and lower regions of the small intestine are similar in appearance although they possess different functional capacities. An extremely intriguing question, still largely unanswered, is that of the morphological basis for these differences. Although some of the transport processes, such as those involving membrane carriers, may be impossible to study histologically, others may be followed directly or indirectly by morphological methods. If transport is intimately associated with specific enzymatic reactions, histochemical studies would be valuable; if a membrane alteration (such as pinocytosis) is involved

in absorption, definite morphological changes should be found with the electron microscope. As the new techniques of histochemistry, electron microscopy, and autoradiography are applied to this problem of the correlation between structure and function, some important answers to the question will undoubtedly emerge.

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CHAPTER 2

Methods

HISTORICAL

The history of the development of techniques for the study of intestinal absorption reflects the history of medical sciences. Observations in the seventeenth and eighteenth centuries on gross morphology indicated that the cloudy appearance of the lymphatics resulted from absorption of fat (see review of Gage and Fish[34]). This was confirmed and considerably extended in the nineteenth century by histological studies of the intestinal epithelium under various conditions. Serious physiological investigation of absorption developed slowly during the last century. Ninety-eight years ago Thiry[88] created a new era in physiological investigation by the description of a surgically prepared intestinal fistula which permitted quantitative experiments on absorption in an unanesthetized animal. This novel method was developed in the physiological laboratory of Ludwig in Vienna, one of the world centers of physiology at the time.

Although quantitative methods began to be applied to this problem, insight into the mechanisms of absorption had to await the advances in organic chemistry, physical chemistry, and enzymology which came in great profusion during the latter half of the nineteenth century. With these advances came further experimentation and controversy about the extent to which absorption could be explained by the laws of simple diffusion. Reid, Heidenhain, and Cohnheim believed that diffusion

could not explain absorption, that other forces ("physiological activity") must be involved. On the other hand, Höber, and later Goldschmidt, believed that osmosis could explain most phenomena of absorption. To support their contentions these workers developed more refined and ingenious methods of investigation. Cannulation of intestinal lymphatics and portal vein helped to decide the route of absorption of various substances. Cori[22] introduced quantitative methods for study in small animals and Höber[41] and Verzár[93] improved the "loop" method in anesthetized animals.

The recent invasion of cellular physiology by biochemically oriented investigators is illustrated by the fact that a number of the modern *in vitro* methods were developed in, or in collaboration with, departments of biochemistry. The first well-oxygenated *in vitro* preparation was developed by Fisher and Parsons[32] in the biochemistry department at Oxford. The tissue accumulation method was biochemically inspired.[3, 23]

A powerful, new tool for the study of absorption is the electron microscope. Aspects of absorption associated with morphological changes such as lipid[69] and protein[17] absorption have been re-examined in recent years and greater insight has been gained into the cellular changes that occur during absorption.

IN VIVO METHODS

Permanent Intestinal Fistula

In 1864 Thiry[88] described a method of surgically preparing a blind loop of intestine, open at one end onto the abdominal wall. A test solution could readily be introduced into the loop and samples withdrawn at any time for analysis. Figure 12 shows a reproduction of the original engraving in Thiry's paper.

Twenty-four years later, Vella[91] in Bologna modified the Thiry fistula to allow both ends of the isolated intestine to open onto the abdominal wall (Figure 13). Such a preparation allowed simplified rinsing of the loop and permitted introduction of test solutions at one end and collection at the other. In addition, Vella used the fistula technique for the cecum and colon.[92]

The primary problem with these two methods was the difficulty in preventing leakage of solutions from the fistula. In an attempt to overcome this disadvantage, Gumilewski[38] placed rubber balloons in both ends of a Thiry-Vella fistula. This general procedure was used by Nagano[61] and White and Rabinowitch.[95] A balloon placed within the loop, however, tends to be drawn in or expelled by peristalsis and

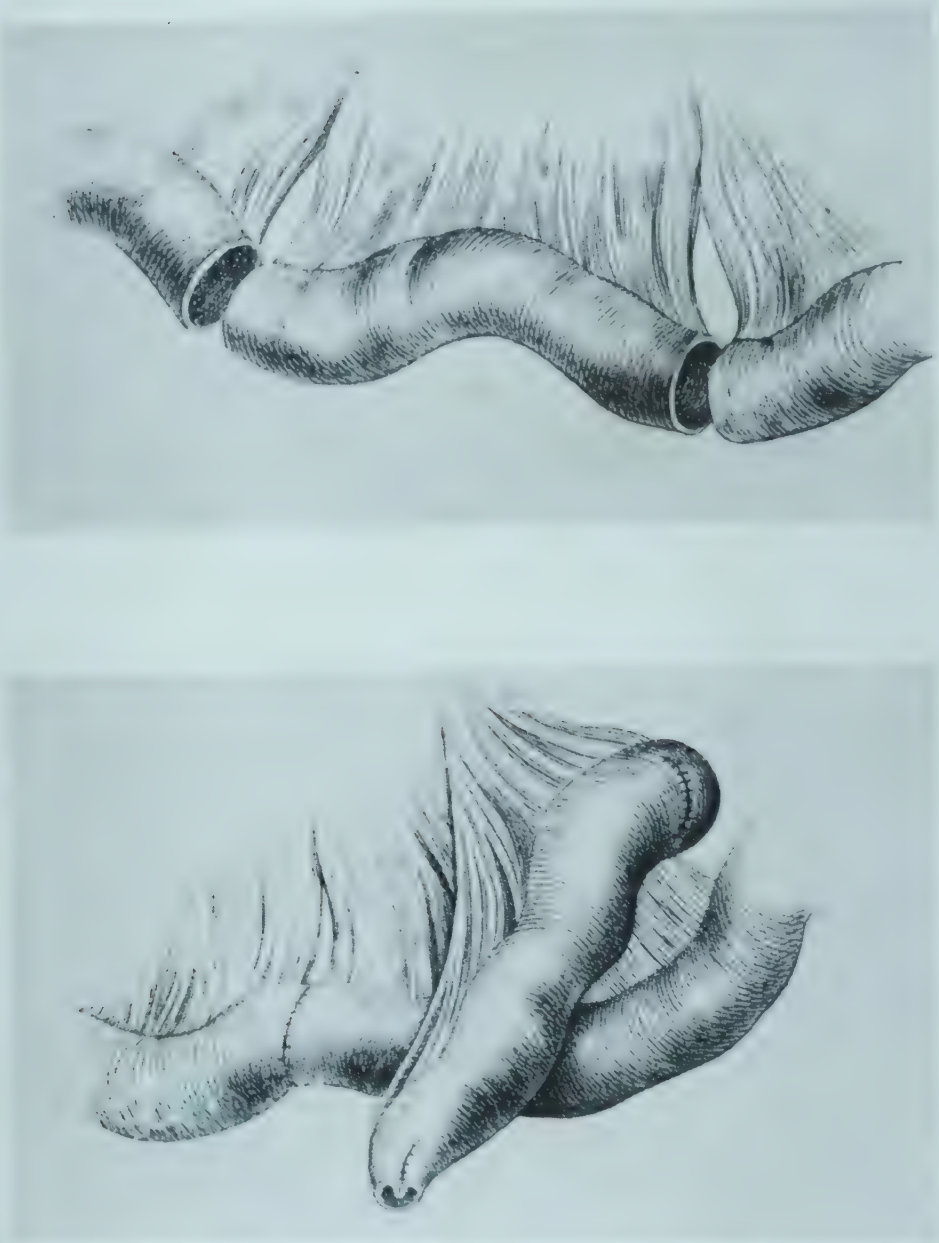

Figure 12. Thiry loop. Small end of the isolated loop in the foreground (lower panel) is in the process of being closed, the opposite end being attached to an opening in the abdominal wall. (Thiry: Sitz. d. Akad. Wien, Math.-Natur. Kl. I. *50*, 1864.)

the system often leaks. In 1932 Johnston[15] described a modified Thiry loop into which a catheter could be firmly anchored with two balloons, one just inside and another outside the abdominal wall. This preparation was used by workers at the University of Pennsylvania[71, 15, 29] and stimulated renewed interest in intestinal fistulae. These preparations are still useful and have been employed in recent years for studies of amino acid[19, 68] absorption.

Occasionally Thiry-Vella loops are available in humans as a result

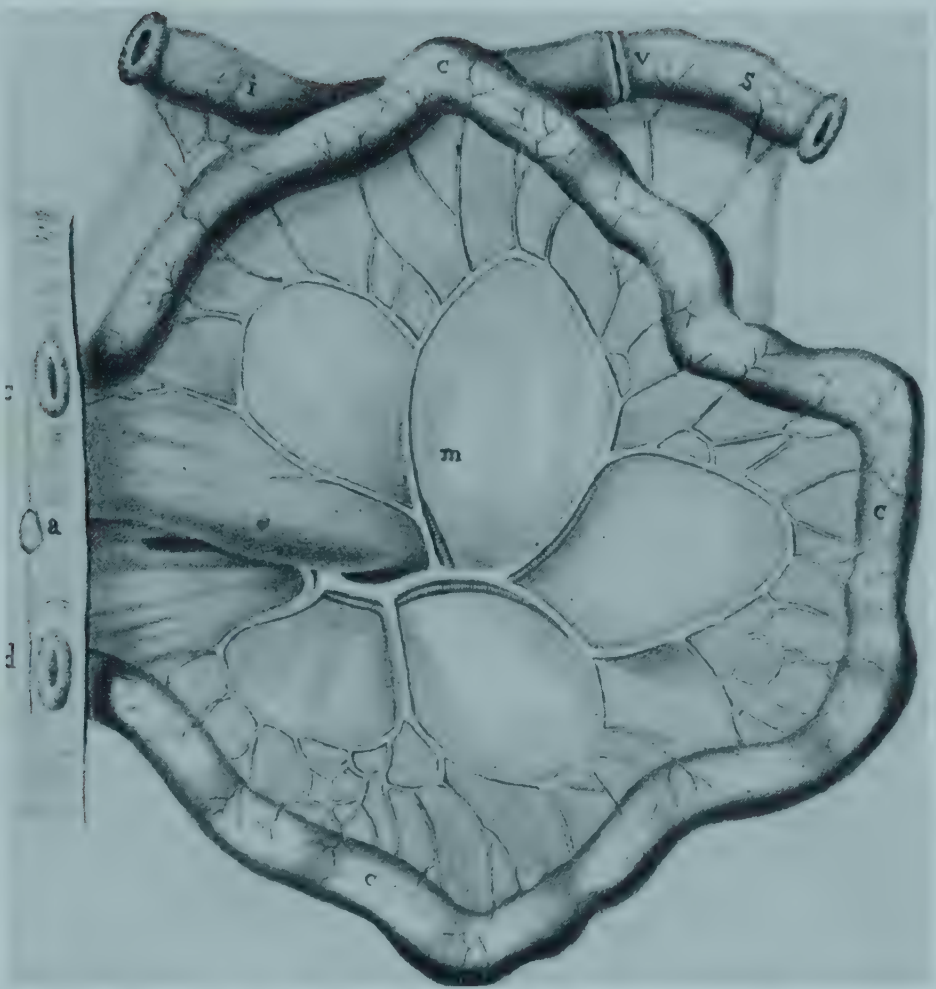

Figure 13. Vella loop. The isolated loop of intestine (c) is attached to openings in the abdominal wall at (c) and (d). The remaining intestine is represented by (i) and (s) which are connected by an end-to-end anastomosis at (v). (Vella: Untersuch. Naturl. Mensch. u. Thiere, *13*, 1888.)

of some unusual surgical complication of an intestinal disorder. Kuroda and Gimble[50] and Orten[66, 67] have studied amino acid absorption in a man with a Thiry loop.

Cannulation of the Portal Vein

Sampling of blood from the portal vein was carried out by Claude Bernard, but chemical methods were so inadequate that little useful information could be obtained. Later, the method was used to establish the portal route for sugar[94] and amino acid[90] absorption but the method did not lend itself to repeated sampling. It was London[54, 55] and his school in Leningrad who popularized the method by developing an elegant permanent preparation for portal vein sampling. This so-called London cannula was a metal cannula surgically fixed in close proximity

to the portal vein or other large vessel in such a manner that a sharp needle could be introduced through the cannula into the vessel for sampling. The surgical difficulties are such that relatively few investigators have utilized this method. Dent and Schilling,[30] however, used this method with considerable success in their important studies of amino acid absorption. More recently, Shoemaker and his collaborators[82] have developed methods of introducing indwelling catheters into a number of vessels, including the portal vein. An example of the results obtained from this method is given in Figure 36 (Chapter 4).

Cannulation of a mesenteric vein in anesthetized animals has recently been used by a number of workers. A vessel is chosen which drains a small segment of intestine which in turn is isolated from the rest of the small intestine by ligatures. If a sugar or amino acid is placed in the lumen of the intestinal segment the absorbed products can be collected quantitatively in the venous blood draining the loop. This method was employed, recently, in the study of the chemical alterations in sugars[5, 48, 49] and amino acids[57, 63] during transit across the epithelial cell.

In patients with portal hypertension the vascular collaterals develop, especially the abdominal anastomotic vessels, permitting sampling of portal blood in unanesthetized human subjects. Several studies of intestinal absorption have been carried out on such individuals.[81, 7, 9]

Cannulation of the Lymphatics

The thoracic duct is the lymphatic vessel most commonly cannulated because of its size and accessibility. Intestinal lymph drains chiefly into the left thoracic duct but probably small collaterals communicate with the duct on the opposite side and there are some lymphatic–blood vessel anastomoses. The presence of collateral vessels[4, 10, 18, 52] probably accounts for the fact that ligation of the left thoracic duct does not appreciably reduce the rate of intestinal absorption of triglycerides.[4, 52] An interesting example of the lymphatic cannulation method is a study by Comline, Roberts, and Titchen[20] who showed that, following colostrum feeding to a newborn calf, as much as 1.2 gm. of unaltered colostrum proteins could be collected from an intestinal lymphatic in a period of ten minutes.

An important advance was made by Bollman, Cain, and Grindlay,[18] who succeeded in developing a method for cannulation of the thoracic duct in the rat. This opened the way for extensive research on lipid absorption in this animal. Numerous examples of data obtained with this method are given in Chapter 7.

Lymphatic fistulae in human beings have been studied for years. Munk and Rosenstein in 1891[59] reviewed a not-inconsiderable earlier

literature before reporting their own extensive experiments on a patient with elephantiasis who had a fistula draining much of the intestinal tract. Ahrens and his collaborators at the Rockefeller Institute recently studied an interesting patient with a communication between an intestinal lymphatic and the renal pelvis.[12] About 20 per cent of the intestinal lymphatic drainage could be collected in the urine. In recent studies, Bierman et al.[8] cannulated the thoracic duct in the neck of human subjects.

The Cori Method

An important method for the study of absorption *in vivo* was developed by Cori.[22] In this method a measured volume of solution was introduced directly into the stomach of an unanesthetized rat by stomach tube. At the end of the experimental period the entire gastrointestinal tract was washed out and the unabsorbed contents analyzed. This method combined the theoretical advantages of studying absorption in the unanesthetized animal with the practical advantage of utilizing large numbers of small animals for quantitative work. It was with this method that the specificity of sugar absorption was first clearly established.[22]

The addition of a soluble nonabsorbable marker to the test solutions has increased the usefulness of the Cori method. Reynell and Spray,[74] using phenol red as a marker, were able to determine gastric emptying and intestinal transit times during periods of absorption. They showed that, with very low glucose loads, the rate of absorption was determined mainly by gastric emptying time,[75] while with higher loads the rate-limiting factor was intestinal absorption.

The Balance Method

This method has been extensively used for absorption studies with human subjects. The oral intake and fecal output is carefully measured and the difference between the two is taken as a measure of absorption (or excretion). Although the method has definite limitations for organic substances, because of bacterial degradation in the colon, it has been most useful for inorganic ions. An example of this technique is given in Figure 79 in Chapter 7.

Tied Loops of Intestine in the Anesthetized Animal

This was one of the first and most generally satisfactory methods used in this field. It has been used extensively by a great many workers, especially by Höber[41] and Verzár.[93] It is possible to place different substances in consecutive tied loops in the same animal and measure disappearance of the test substance from the lumen. One may also use the same loop for more than one absorption period. A detailed descrip-

tion of the method is given in Verzár's book.[93] It was with this general method[48] that Visscher and his colleagues carried out their extensive investigations on fluid and electrolyte absorption.

Circulation of Fluid Through a Loop of Intestine

A modification of the previous method is perfusion of fluid through a loop of intestine with or without recirculation of fluid. In 1917, Sols and Ponz[85] described one such method (Figure 14). This general pro

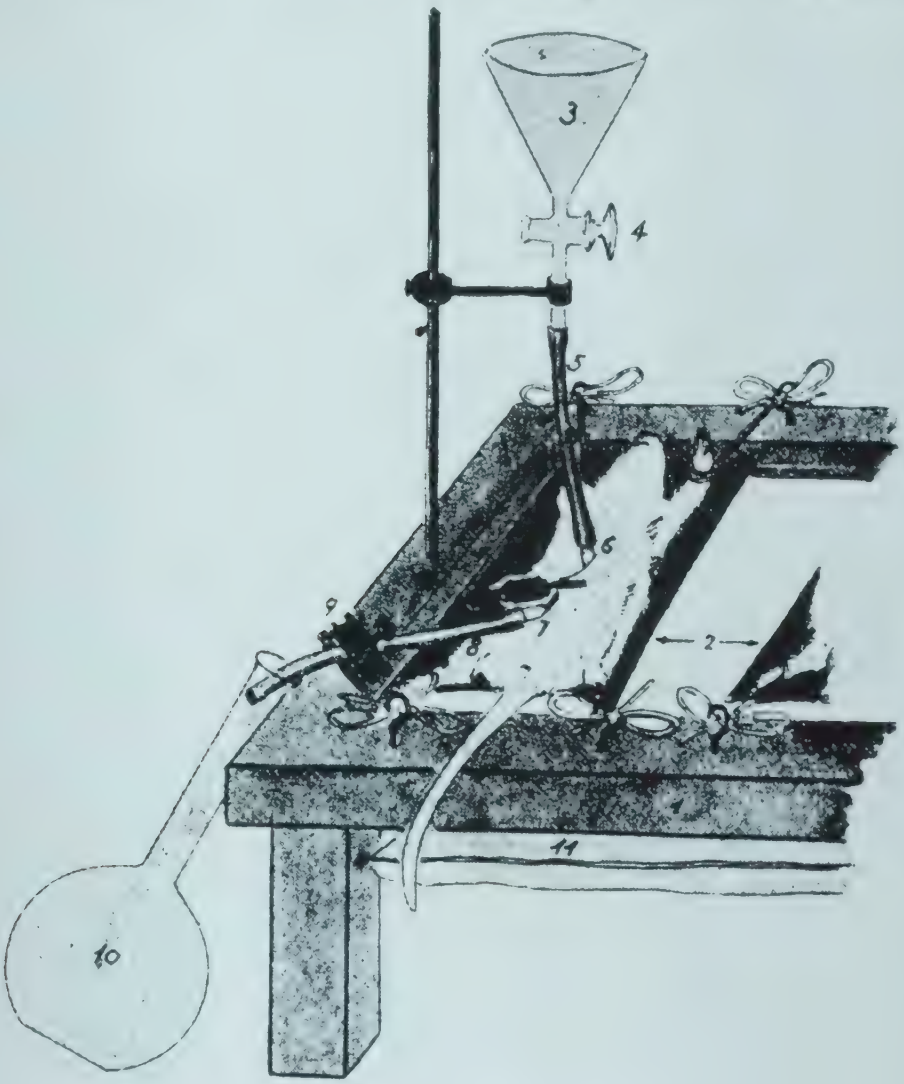

Figure 14. *In vivo* circulation method of Sols and Ponz.

1. Wooden frame of 32 by 28 cm. provided with legs of 20 cm. in length and two stems of 25 cm.
2. Pieces of canvas in the shapes of hammocks for the rats.
3. Funnel with key (4).
5. Rubber tube connecting funnel with cannula of entrance 6.
7. & 8. Cannula and issue tube, the latter provided with a clamp 9.
10. Volumetric flask.
11. Electric pillow.

(Sols and Ponz: Rev. espan. fisiol., *3*, 1947.)

cedure, with modifications, has been used by a number of workers.[33, 44, 80, 42] A similar method was used by the Bethesda group[78] in their extensive study of absorption of drugs from the intestine.

Intubation Methods for Humans

In 1934 Miller and Abbott[58] described a method for the rapid intubation of the human small intestine. It involved the use of a double-

Figure 15. Miller-Abbott tube. Longitudinal sections of double-lumen rubber tube. The lumen on the right side is referred to as "A" and the other lumen as "B." 1 indicates a proximal tube communicating with "A"; 2, a proximal tube communicating with "B"; 3 indicates a brass cannula for making the connection between a lumen of the double tube and proximal single tube; 4, thread holding two brass tubes in place; 5, outer wall of double-lumen tube; 6, transverse section of tube; 7, opening into lumen "A"; 8, Rehfuss bucket; 9, rubber bag (condom or finger cot). (Miller and Abbott: Am. J. M. Sc., *187*, 1934.)

lumen rubber tube, to the distal end of which was attached a collapsible rubber balloon which could be distended through one of the lumens (Figure 15). The balloon, when suitably inflated, could stimulate peristalsis active enough to propel it through the entire length of the small intestine in three to four hours. The balloon also obstructed the lumen so that fluid could be aspirated through the second lumen. It was possible to place the tube at any desired location in the gut and aspirate contents before and after feeding various substances. In 1936 Abbott and Miller[1] published a description of a triple-lumen tube which was used to isolate a segment of intestine between two rubber balloons. The contents of the area between the balloons could be aspirated and absorption studies made, uncomplicated by food and secretions from adjacent segments of intestine. This method and its modifications[25, 26, 37, 64, 79, 106] have been very useful in the study of absorption in man.

The most recent intubation method is that introduced by Blankenhorn, Hirsch, and Ahrens[11] and used by them and by Borgström and his collaborators.[14] In this method a thin polyvinyl tube (2.1 mm. O.D.) is allowed to pass through the entire gastrointestinal tract. The tube is then permitted to enter continuously through the nose and leave via the anus at a rate depending on normal peristalsis. By various ingenious devices, sampling can be made at any desired level of the intestine. Transintestinal intubation for periods of one to two weeks is well tolerated and sampling may be effected under conditions which closely approach the physiological state. This method will probably be used more extensively in the future.

IN VITRO METHODS

Early Methods

One of the early studies specifically related to permeability of the intestine was by Jones in 1854[47] in which an isolated segment of racoon small intestine was placed in a solution of oxalate and a calcium solution was passed through the lumen. As a precipitate appeared in the oxalate solution it was inferred that calcium ions had crossed the intestinal wall. A similar type of experiment on the movement of acids across the isolated intestine was depicted in a book by Carpenter in 1865.[16] The first important physiological information obtained with isolated intestine concerned the absorption of fluid across the intestine against a hydrostatic pressure gradient. This observation was made by Reid[72, 73] with an ingenious device which consisted of two compartments between which a sheet of intestine was placed. This device, a prototype of more modern ones used for the study of membrane transport, is depicted in Figure 16.

In 1930, Auchinachie, Macleod, and Magee[6] studied the absorption of sugars with isolated rabbit intestine suspended in a beaker of oxygen

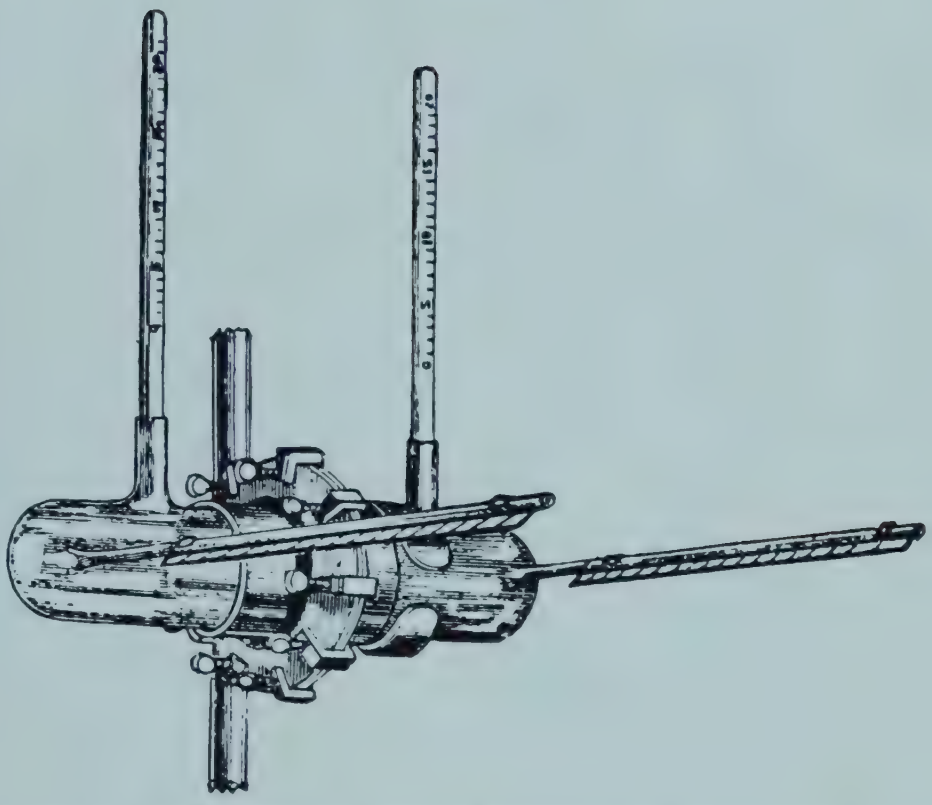

Figure 16. A diaphragm of intestine separating two compartments. A circular segement of intestine (not seen in figure) separates two glass compartments. Capillary tubes attached to each compartment are for recording pressure changes. (Reid: J. Physiol., *26,* 1901.)

ated Ringer's solution. With this preparation they were able to show that while glucose was absorbed much faster than xylose at 37°C., both sugars were absorbed at the same rate at 0°C. This was one of the first indications that some process other than simple diffusion was involved in glucose absorption. This preparation has been used occasionally[40] but is not entirely satisfactory because of inadequate oxygenation of the epithelium.

Perfusion of Isolated Intestine

The intestine perfused with blood or saline solution will function *in vitro* but it is not a simple method. Ohnell[65] has reviewed the previous studies and described his own preparation. Lundsgaard performed a series of studies on sugar transport and metabolism in cat intestine by this method.[56] In recent years the method has been used infrequently.

Circulation Techniques

The first well-oxygenated *in vitro* preparation of the small intestine for absorption studies was described in 1949 by Fisher and Parsons.[32] Figure 17 shows the apparatus for circulating oxygenated Krebs solution on both sides of a segment of rat intestine. Warm Krebs solution was

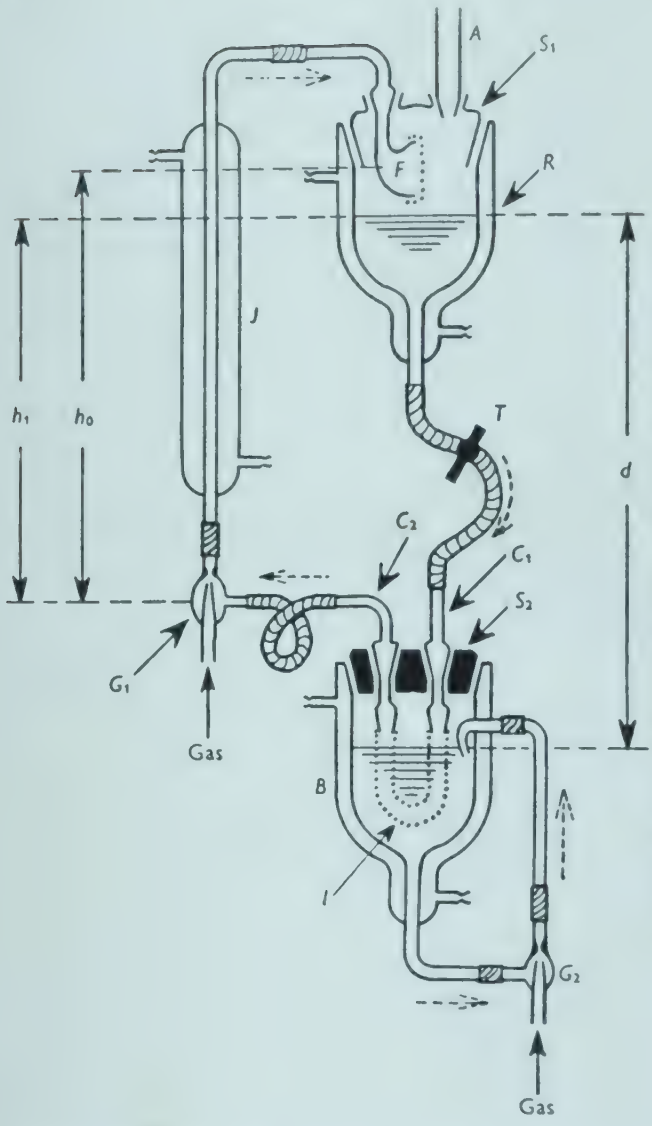

Figure 17. Circulation apparatus of Fisher and Parsons. The inner fluid passes from the reservoir R through a rubber tube furnished with a screw clamp T to the glass tube C_1. The inner fluid passes from C_1 through the lumen of the intestine I to a second glass tube C_2. From C_2 fluid passes to G_1 which is a small gas-injector which introduces a stream of gas bubbles (5 per cent CO_2 and 95 per cent O_2) which raises the fluid through the vertical tube J and through the anti-frothing device F back into the reservoir R. A similar oxygenation device G_2 is provided for the lower compartment. With h_1 equals 35 cm., and h_0 equals 40 cm. and the tubes have an internal diameter of 4 mm., circulation rates of 35 to 45 ml. min. are obtained. (Fisher and Parsons: J. Physiol., *110,* 1949.)

passed through the lumen of the intestine with a hydrostatic pressure of 20 cm. of saline. The fluid was returned to the reservoir and oxygenated by bubbles of oxygen rising in a vertical tube. The solution on the serosal side of the intestinal wall was also oxygenated. This preparation was used by Fisher and Parsons[32] and others[70, 2] for the study of sugar and amino acid absorption.

Wiseman[103] has described another apparatus for circulating oxygenated solutions on the two sides of the small intestine (Figure 18.) In this preparation, fluid passes down three segments of intestine and is returned to the reservoir via a single tube provided with an oxygen bubbler. With this method, Wiseman demonstrated active transport of L-amino acids across rat intestine against a concentration gradient. The method was also used by Muto[60] for studies of riboflavin absorption. This has been modified by Smyth and Taylor[84] by the removal of the

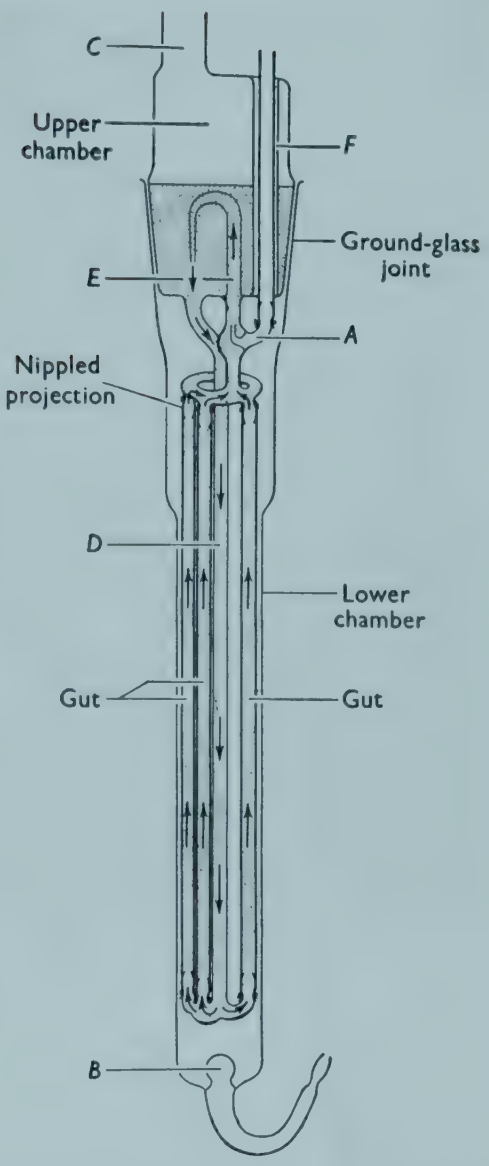

Figure 18. Circulation apparatus of Wiseman. The upper chamber, which contains the inner fluid and carries the intestinal segments, fits into the lower chamber (containing 50 ml.), is open at C, has a capacity of about 50 ml. and a diameter of about 4 cm. Three segments of intestine each 20 cm. long are attached. Fluid moves from the upper chamber down tube D and rises through the lumen of the three segments of intestine and then returns to the upper chamber by means of an oxygen bubbler, A. The serosal fluid (25 ml.) in the lower compartment is oxygenated through bubbler B. (Wiseman: J. Physiol., *120*, 1953.)

serosal fluid. Fluid and solutes absorbed by the intestine are not diluted by a large serosal fluid volume but allowed to drain from the serosal surface and drop into a test tube at the bottom of the apparatus. This method has also been used by Gilman[36] for studies of fluid and electrolyte absorption.

Lee[53] has recently investigated the route of fluid absorption through the intestinal wall *in vitro*. He cannulated a mesenteric vein and lymphatic vessel and then circulated Krebs solution through the lumen. With 10 mm. Hg lavage pressure, 85 per cent of the absorbed fluid appeared in the lymphatic vessel, 11 per cent in the mesenteric vein, and only 4 per cent diffused across the serosa. Occlusion of vessels led to elevated hydrostatic pressure within them and inhibition of fluid absorption.[53, 35] These observations are probably applicable to other *in vitro* methods.

Darlington and Quastel[28] have used another *in vitro* preparation which has the advantage of simplicity of construction (Figure 19). A considerable number of published studies have come from the Montreal laboratory using the method. A modification of this method combined with the use of everted hamster intestine has been described by Wilson.[97]

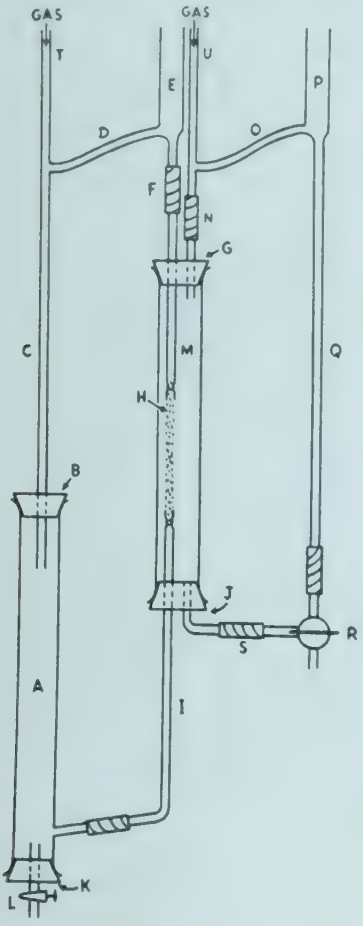

Figure 19. Circulation method of Darlington and Quastel. The inner solution passes from a cylinder A, through C and a connecting arm D, into a small reservoir E which is open to the atmosphere. From E the fluid passes through F, which connects with the intestinal segment H, through I and back into the cylinder A. The lower opening in cylinder A is closed by a rubber stopper through which passes the glass tubing of a stopcock. This stopcock serves to drain the inner fluid at the end of the experiment. The volume of the inner solution is approximately 100 ml.

The outer solution, which in cylinder M bathes the outer surface of the intestinal segment H, passes from the cylinder through N and the connecting arm O into the small reservoir P which is also open to the atmosphere. From P the fluid passes down glass tubing through a three-way stopcock into the cylinder M. Tubes T and U are for introduction of oxygen which circulates and oxygenates the fluids. The three-way stopcock R is used to drain the outer fluid at the end of the experiment, which is collected and measured. The volume of the outer solution is approximately 110 ml. (Darlington and Quastel: Arch. Biochem., *43*, 1953.)

Everted Sac Method

The everted sac method of Wilson and Wiseman[101] involves the use of small segments of intestine, turned inside out, filled with fluid, and tied at both ends. Its primary virtue is its simplicity, the equipment involving only an Erlenmeyer flask and a water bath. In addition, the small serosal volume results in large concentration changes in transported substances. Figure 20 shows a sac of everted hamster jejunum. The sac is distended with fluid which separates the individual villi and allows circulation of oxygenated Krebs solution between them. The very high oxygen requirement of epithelial cells[31, 102] necessitates special precautions for adequate oxygenation (see review by Wiseman[105]).

A few comments on this method may be useful. The original method described the introduction of a bubble of oxygen into the sac for the pur

Figure 20. Everted sac method of Wilson and Wiseman. Tied sac of everted hamster jejunum containing 1 ml. of fluid. X 3. (Wilson: 12th Ann. Conf. Nephrotic Syndrome.)

pose of oxygenation. It has been found, empirically, that this procedure is unnecessary, presumably because of the low oxygen utilization of the muscularis and connective tissue and their minor role in the absorption process. The degree of distention of the sac is probably important, as movement of fluid from serosal to mucosal side, due to hydrostatic pressure, greatly inhibits sugar transport[97] (at least in the hamster). For studies of absorption, the sac should be filled so that additional uptake of fluid during absorption is possible. In this regard, the hamster, rat, and small rabbit have been found most satisfactory; the smooth muscle of the guinea pig and frog intestine contract so vigorously that fluid is forced out of the everted sac during incubation.

Because of its simplicity, the everted sac method has been used for the study of absorption of a variety of substances: sugars,[99] amino acids,[62, 104] fatty acids,[46] triglycerides,[86] nucleotides and their derivatives,[77, 100] bile salts,[51] cholesterol,[83] vitamins,[39, 89, 87] inorganic salts,[76, 96, 98, 21] and proteins.[27] The main disadvantage of the method, difficulty

in obtaining more than one serosal sample, may be overcome by Johnston's suggestion[16] of tying to the end of the sac a polyethylene tube through which multiple samples may be obtained with a fine needle.

A useful modification of the sac method was suggested by Crane and Wilson.[24] This "test-tube method" combines some of the features of the simpler sac method with the additional feature of the intestine being tied to a cannula from which frequent serosal samples may be obtained (Figure 21). With this method a number of different solutions may be tested with a single segment of tissue.

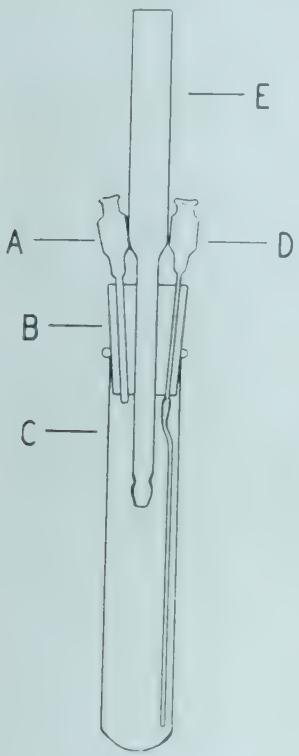

Figure 21. Test tube method of Crane and Wilson. *A*, No. 15 needle, blunted; *B*, No. 1 rubber stopper with one hole; *C*, plastic (Lusteroid) centrifuge tube, 15 ml. capacity (15 x 95 mm); *D*, No. 21 needle with 8 cm. length of polyethylene tubing; *E*, Pyrex tube, upper portion 6 cm. long and 10 mm. outer diameter; lower portion 5 cm. long and 5 mm. outer diameter, with a cannula tip to which the open end of the sac is attached. (Crane and Wilson: J. Appl. Physiol., *12*, 1958.)

Tissue Accumulation Method

In 1956, Agar, Hird, and Sidhu[3] studied the accumulation of L-histidine by small segments of rat intestine (Figure 22). They found that this accumulation process occurred with L- and not D-histidine and was inhibited by DNP, cyanide, and other L-amino acids. This method has proved extremely useful because it is simple and reproducible. Hird and his associates, as well as others, have used it effectively for the study of amino acid transport.

Crane and Mandelstam[23] have used this method to advantage in sugar absorption. They also described a preparation of intestinal villi which showed the same phenomena of tissue accumulation.

A logical extension of the *in vitro* methodology is the isolation of single epithelial cells. This would appear to be quite feasible as methods

are available for separating individual cells from a variety of organs of the animal body. The use of suspensions of such cells, free of connective tissue and smooth muscle, may be of considerable value in transport or metabolic studies. Perhaps means may be found to separate the individual cell types which make up the intestinal epithelium and discover the function of such cell types as the argentaffine and Paneth cells about which so little is known.

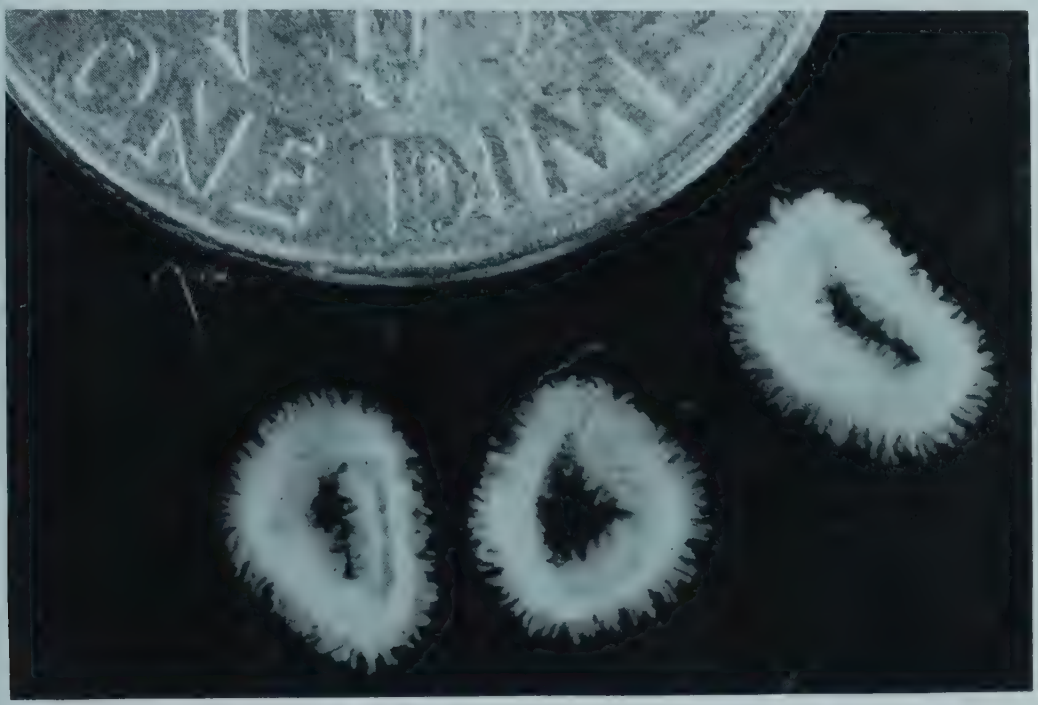

Figure 22. Tissue accumulation method of Agar, Hird, and Sidhu. Small rings 1 to 5 mm. in width were cut from everted intestine of the hamster. Dime shown for comparison. Approximately x 7.

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CHAPTER 3

Mechanisms of Absorption

For over seventy years there has been a debate about the extent to which the small intestine behaves as a passive semipermeable membrane and to what extent it performs "secretory" or absorptive work requiring energy from cellular metabolism. Heidenhain and Reid were proponents of the view that "vital" cellular activity was essential, while others such as Höber (and later Goldschmidt) believed that the laws of simple diffusion could account for most absorptive phenomena of the intestine. As in many such debates, both views were later shown to be partially correct, although special absorptive mechanisms probably play a more important role than diffusion. The purpose of this chapter is to give examples of the different mechanisms involved in intestinal absorption.

In the classification of permeability phenomena commonly employed, a primary division is made between passive diffusion and special mechanisms (e.g., active transport, facilitated diffusion, and pinocytosis). The subdivision of the two major categories depends somewhat on the author. As the physiochemical mechanisms for some of the processes are poorly understood, classification in these cases will understandably be rather arbitrary. Passive diffusion is the best understood permeability phenomenon, at least insofar as it applies to certain artificial systems and a few living membranes such as those of certain plant cells,[11, 84, 85] the erythrocyte,[59] and the capillary.[71, 89] Simple diffusion in some living cells, however, is not as simple and unambiguous as it may be in artificial

systems, as shown by the high temperature coefficient sometimes found for diffusion through living membranes[21] and by the effect of cellular metabolism on diffusion processes in certain tissues. Living cells do not possess cellophane membranes and many of the permeability characteristics of cell membranes are modified by properties inherent in their complex structure and by the metabolic reactions maintaining them.

Considerable progress has been made in recent years in the direction of elucidation of absorptive mechanisms of the intestine, and a classification of our present knowledge might be useful. If one defines precisely the meaning of the terms used and realizes the limitations of the system, a classification can form an important series of working hypotheses around which existing information can be organized. Inconsistencies between data and hypotheses may stimulate further research.

PASSIVE DIFFUSION

Diffusion will be discussed first because of its simplicity and not because of its primary importance in intestinal absorption. Historically, diffusion was the first mechanism studied in detail and deviations from the laws of simple diffusion led to the discovery of the various special absorptive mechanisms. Although active transport and other special mechanisms are largely responsible for absorption of most nutrients, simple diffusion appears to be the mechanism of absorption of such important substances as some water-soluble vitamins, some nucleic acid derivatives, and many lipid-soluble substances.

Pore Route

Diffusion across thin, artificial membranes has been extensively investigated; the rate of movement of a given substance is proportional to the concentration difference across the membrane. The process is quantitatively described by the Fick equation:

$$\frac{ds}{dt} = K\ (A)\ \frac{(\text{external concentration} - \text{internal concentration})}{\text{thickness of membrane}}$$

in which ds/dt is the rate of movement of solute, S, across the membrane, A is the area of the membrane, and K is a constant. As the thickness of the cell membrane is usually not known, this factor is commonly combined with the constant, K, to give a new constant, P (permeability constant). The equation then becomes:

$$\frac{ds}{dt} = P\ (A)\ (\text{external concentration} - \text{internal concentration}).$$

From this may be derived a variety of different equations applicable to specific experimental conditions.[52] The simple Fick equation is applicable only to nonelectrolytes; a somewhat different treatment is given to water[52, 55, 90, 117] and to electrolytes.[116]

The application of Fick's equation to diffusion across the intestinal epithelium is complicated by the particular anatomical arrangement of the cells. The surface area across which diffusion occurs is not easily calculated, as movement occurs across three permeability barriers (two cell membranes and a basement membrane). The area of the luminal border of the cell, made up of microvilli, is at least ten times greater than either of the other two membranes. It is conceivable that one membrane may be the effective permeability barrier for water-soluble substances and another for lipid-soluble substances. Thus, it is difficult to substitute a value for A in the diffusion equation. It is also important to realize that the epithelial cell is about three orders of magnitude thicker than a plasma cell membrane (compare, for example, the thickness of the erythrocyte membrane [0.01μ] and the epithelial cell [10 to 15μ]). As mentioned above, the common measure of permeability, the permeability constant P, includes the membrane thickness but certain quantitative studies, especially those involving bulk flow of water, require a knowledge of thickness.

Simple diffusion is undoubtedly a very important pathway for absorption from the small intestine and there are a number of good examples. Figure 23 shows an experiment by Verzár[120] in which the rate of sorbose absorption from loops of rat intestine *in vivo* was measured at

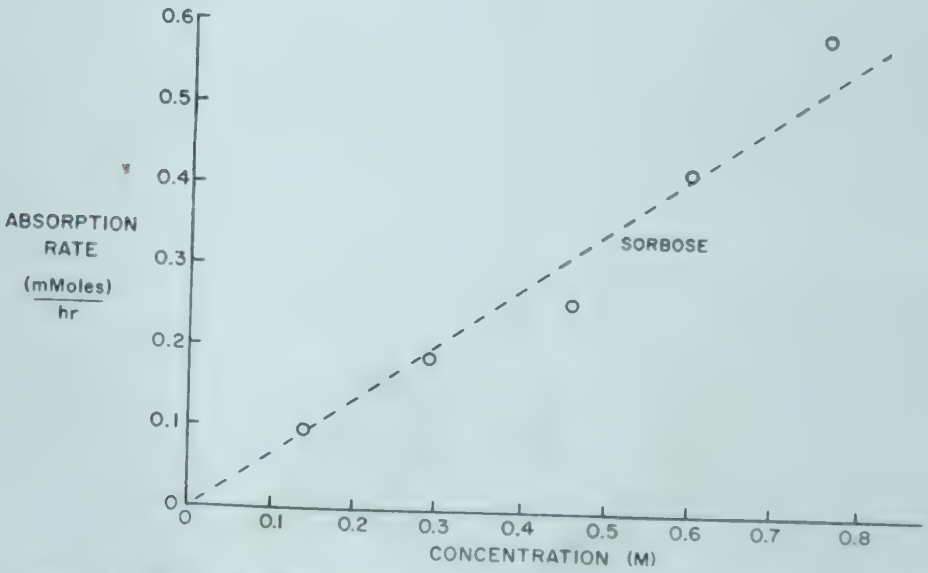


Figure 23. Effect of concentration on absorption rate of sorbose. Sorbose was placed in tied loops of rat intestine *in vivo* and rate of disappearance from loop was measured. (Drawn from the data of Verzár: Biochem. Ztschr., *276*:17, 1935.)

different concentrations. Over a wide range the absorption rate was directly proportional to the concentration in the lumen, the concentration in the blood being virtually zero. Further evidence to suggest that sorbose is absorbed by diffusion includes these facts: (1) It is not absorbed against a concentration gradient.[135] (2) Its absorption rate is not inhibited by phlorizin or dinitrophenol.[8] (3) Other sugars do not compete with it for absorption.[20, 28]

A variety of other examples may be cited in which Fick's law applies. Urea, for example, has been studied by a number of workers and its absorption rate is a linear function of concentration difference.[36, 40, 72] Furthermore, it diffuses in both directions across the intestinal epithelium. Other substances showing similar behavior include mannose,[120] xylose,[120] fructose,[96, 135] erythritol,[40] and malonamide.[40]

A linear relation between absorption rate and concentration gradient, while consistent with diffusion, does not exclude absorption by enzymatic mechanisms (or by one involving some type of adsorption reaction). A similar result could be obtained in a transport process mediated by enzymes, provided the substrate concentration was well below the K_m (Michaelis-Menten constant). Fortunately for the experimenter, many of the special mechanisms (not resulting from diffusion) are completely saturated at concentrations of 10 mM. or less and this confusion does not often occur.

As early as 1899 Höber[38] showed that disaccharides were absorbed more slowly than monosaccharides and concluded that diffusion was an important mode of absorption by the intestinal epithelium. A more instructive example of the same type is taken from a later paper by Höber and Höber[40] in which a comparison was made between absorption rates of amides and their molecular volume (Table 4). They inferred that the apparent pore size was probably approached by succinamide since a relatively small increase in volume caused a disproportionate decrease in absorption rate.

A similar study with a series of sugars is shown in Table 5. Active transport of these sugars against a concentration gradient does not occur[135] and presumably they are absorbed by diffusion. Inulin and

Table 4. Effect of Molecular Volume on Intestinal Absorption

SUBSTANCE	MOLECULAR VOLUME	ABSORPTION RATE PER CENT
Acetamide	69	73
Lactamide	99	40
Succinamide	126	5

Taken from Höber and Höber: J. Cell. & Comp. Physiol., *10*:401, 1937.

starch are not absorbed and compounds with a molecular weight from 200 to 400 are just able to penetrate the cell. In 1957 Curran and Solomon[17] estimated the effective pore radius as 36 A°, making certain assumptions. A more recent estimate by Lindemann and Solomon,[77a] using an entirely different technique, gives a value of 4 A° which is of the order of magnitude presumed for most other cell membranes.

Table 5. Effect of Molecular Size on Rate of Absorption of Sugars

CARBOHYDRATE	MOLECULAR WEIGHT	RADIUS OF EQUIVALENT SPHERE (A°)*	RATE OF MOVEMENT FROM MUCOSAL TO SEROSAL SIDES (μMOLES/100 MG. WET WT./HR.)†
Polysaccharide (starch)	50,000±		0
Inulin	5,000	14.8	0
Disaccharide (lactose)	342	4.4	0.5
Hexose (mannose, sorbose)	180	3.6	1.9
Pentose (ribose, arabinose)	150		2.2
Triose (glyceraldehyde)	90		4.5

* The radius of a particle that would show the same diffusion coefficient in solution as that actually observed for the particular molecule.[88]

† The initial concentration gradient for the four sugars was 0.3M while that for inulin and starch was 1 per cent. Calculated from data of Wilson and Vincent: J. Biol. Chem., *216*:851, 1955.

Lipid Route

The classical studies of Overton[84-86] and later Collander and Bärlund[11] established the fact that while the rate of penetration of small water-soluble molecules could satisfactorily be explained by diffusion through water-filled pores in the membrane, the rate for a large group of substances could be explained only by their passage through lipid of the cell membrane. The rate of penetration of diethylurea, for example, is more than ten times that of urea, and the lipid solubility of the first is about ten times that of the second. These general observations have been confirmed and extended to almost all living cells. Clear examples of this type of diffusion may be found in intestinal absorption. Table 6 gives examples in which lipid solubility appears to be the important factor in determining absorption rate.

There are many drugs whose molecular size would not be favorable to passage through pores, but because of their lipid solubility, absorption is fairly rapid. An excellent example of this type is taken from the study of Schanker[103] on barbiturate absorption by the rat colon (Figure 24). In this example the rate of absorption increased despite an increase in

Table 6. Effect of Lipid Solubility on Absorption

SUBSTANCE	MOLECULAR VOLUME	DISTRIBUTION COEFFICIENT OIL/WATER	ABSORPTION PER CENT
Succinamide	103	0.0049	82
Lactamide	99	0.00058	67
Malonamide	104	0.00008	13

Taken from Höber and Höber: J. Cell. & Comp. Physiol., *10*:401, 1937.

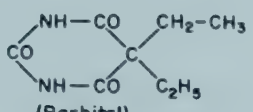
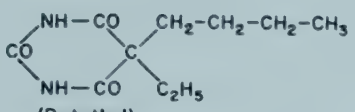
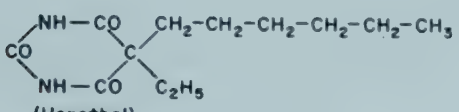
COMPOUND	CHLOROFORM / WATER PARTITION COEFF.	% ABSORBED
 (Barbital)	0.7	12
 (Butethal)	11.7	24
 (Hexethal)	>100	44

Figure 24. Effect of lipid solubility on absorption of a series of barbituric acid derivatives. (Drawn from the data of Schanker: J. Pharmacol. & Exper. Therap., *126*:283, 1959.)

molecular weight, a result that is inexplicable on the basis of simple diffusion through small water-filled channels. Hexathal, because of its much greater lipid solubility, is absorbed about four times as fast as barbital.

Weak Electrolytes

It has been known for many years that cellular permeability to weak electrolytes may be dramatically affected by relatively small changes in the pH of the suspending medium. The rate of penetration of weak acids and weak bases is enormously increased by altering the pH of the external environment in the direction of the pK of the compound. The explanation for this behavior is in the fact that the cell membrane is much more permeable to the undissociated molecule than to the ionized form, especially when the un-ionized form is lipid soluble. Examples of this behavior may be cited for carbonic acid,[48, 49] H_2S,[83] ammonium salts,[50, 53] and dyes.[39] Although this phenomenon is of general significance in all cells, only recently were good examples described in the case of the intestinal epithelium.[42, 43, 103, 104, 106, 108]

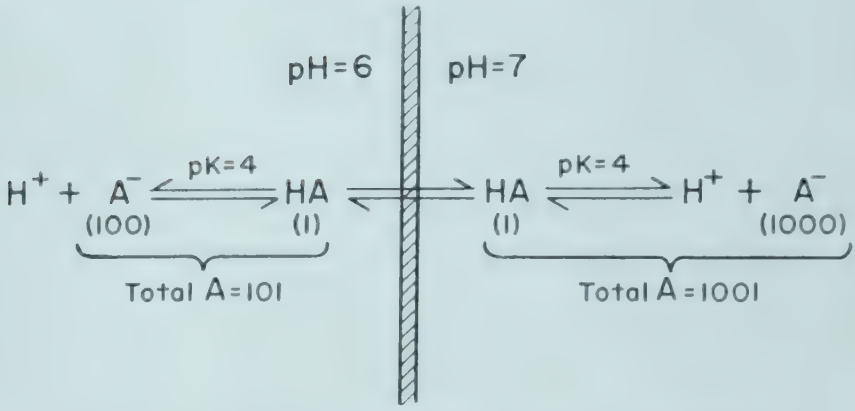


Figure 25. Mechanism of membrane penetration by weak acid.

Let us assume that a membrane is completely impermeable to the dissociated acid, A^-, while it is freely permeable to the undissociated species HA. With a pK of 4 for the reaction $H^+ + A^- \rightarrow HA$, let us now change the pH of the left-hand side to pH 6 by the addition of a small amount of HCl or other strong acid (see Figure 25). There is now more HA formed on the acid side. This upsets the equilibrium across the membrane and more HA crosses to the right-hand side. The net result is the reduction in the total amount of HA plus A^- on the left-hand side and a rise on the opposite side.

Figure 26 shows two examples of this phenomenon in absorption from the intestine. Absorption of the weak acid, 5-nitrosalicylic acid, is

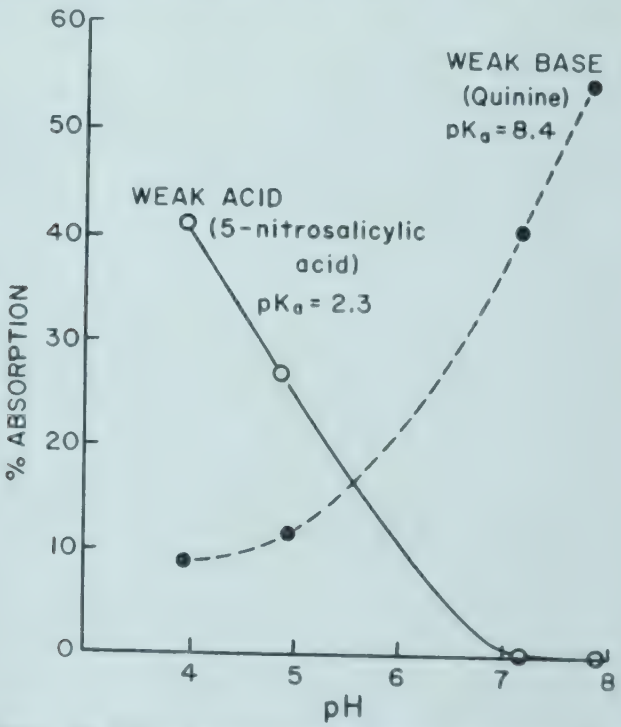


Figure 26. Effect of pH on absorption of weak electrolytes. (Hogben et al. J. Pharmacol. & Exper. Therap., *125*, 1959.)

much more rapid in acid solution than when neutral or in alkaline solution. Appreciable absorption occurs only when the pK is approached and a significant fraction of the molecules are un-ionized. Likewise, with the weak base, quinine, the un-ionized species is absorbed more rapidly than the ionized one. This principle has some practical application in drug therapy, as environmental pH can at times be manipulated so that absorption is facilitated or inhibited. Still another method of promoting absorption of a compound is to remove the charge of a carboxyl group by formation of an amide or ester, or otherwise to remove ionizable groups.

Intestinal Epithelium as a Barrier to Diffusion

Because many electrolytes and nonelectrolytes of various sizes are absorbed by the intestine, a superficial impression might be obtained that the epithelium is very permeable and exceedingly unselective. In the light of present knowledge it appears more likely the intestine is a relatively impermeable structure with a variety of highly specific transport systems for biologically important substances.

The intestine is impermeable to most particulate material such as bacteria, starch grains, and emulsions of most hydrocarbons.[121] There is recent evidence demonstrating particulate absorption in the adult animal under certain conditions,[3, 101] but its quantitative significance has yet to be assessed. The question of particulate absorption during triglyceride absorption[1, 88] is still unsettled and is discussed in detail in Chapter 7. Suffice it to say, in the present context, that if absorption of particulate lipid occurs it must be an exceedingly selective process, as other substances of large molecular dimensions[61] do not penetrate the intestinal epithelium to any appreciable extent.

The adult intestine is likewise impermeable to most water-soluble compounds with high molecular weights (including starch, cellulose, inulin, and polyethylene glycol). This last compound, which has a molecular weight of 3000 to 3700, has been studied in man and virtually complete recovery is obtained in the feces after oral administration.[107] Hogben[41] stated: "Water-soluble and lipid-insoluble solutes with a molecular weight somewhat larger than 100 are poorly absorbed if at all." Although the value of 100 may be rather too small, the statement emphasizes the relative impermeability of the intestinal epithelium. The short period of protein absorption which occurs in fetal and newborn animals of certain species is a special subject considered in Chapter 10. The adult animal is capable of absorbing intact protein molecules only in antigenic amounts.

Highly ionized compounds comprise another category of poorly absorbed compounds. It has been known for many years that cells are

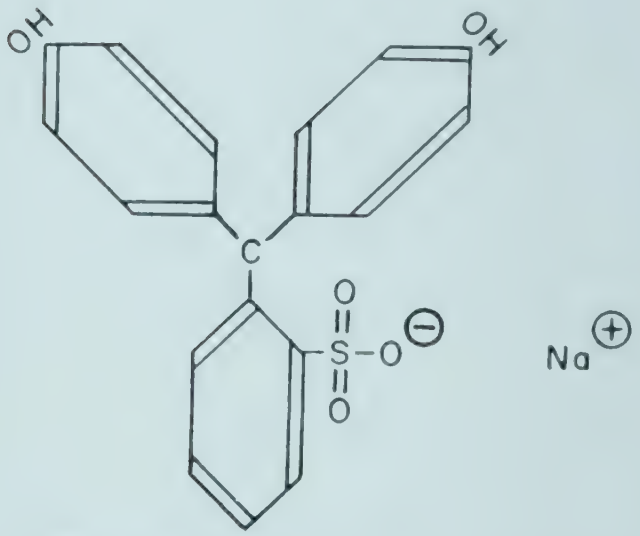


Figure 27. Structure of phenol red at pH 7.

relatively impermeable to most salts of strong acids and bases, especially polyvalent compounds and those of large molecular weight. The physiological basis for the cathartic action of sulfate, phosphate, and citrate is the very low absorption rate of polyvalent ions. Phenol red (phenolsulfonthalein) is an example of a charged molecule of moderate molecular weight (Figure 27) which is not absorbed by the small intestine of the rat. Hogben, Schanker, Shore, and their collaborators[42, 43, 100, 103, 104, 106, 108] have recently published a series of papers illustrating this principle very clearly. Singly charged acids with a pK below 4 and bases with a pK above 9 are poorly absorbed from the small intestine or colon.

The conclusion is that the intestinal epithelium is a highly effective barrier for diffusion of a wide variety of substances, especially those that are charged or of high molecular weight. The apparent exceptions to this generalization probably involve specialized and usually highly specific mechanisms which absorb biologically important substances.

MEMBRANE PERMEABILITY TO WATER

As early as 1935 Jacobs[52] pointed out that osmosis and diffusion are different in principle although the two phenomena show formal similarity. It appears that under some conditions the behavior of water can best be explained by a bulk flow. Hevesy, Hofer, and Krogh[37, 69] found that with frog skin the ratio of the flux rates of D_2O in the two directions was considerably greater than the thermodynamic activity ratio for water across the membrane. Similar discrepancies have been found in other cells.[67, 87, 94, 109, 122]

The concept of bulk flow of fluid can be illustrated in the case of a membrane permeable to water but not to solute molecules (Figure 28). In this figure, taken from the paper of Ussing and Andersen,[118] we may consider what happens at the right-hand side of the water-filled pore where water and solution meet. There will be a tendency for the water to diffuse from higher activity (in pure water) to the lower activity in the solution. This diffusion of water across the dotted line will cause a hydrostatic flow of fluid down the cylindrical pore. This hydrostatic flow will be proportional to the fourth power of the radius (Poiseuille's law) whereas diffusion is proportional to the square of the radius. Pappenheimer[90] and Ussing[67, 119] proposed the ingenious idea of combining Fick's and Poiseuille's equations in a manner that makes it possible to determine the pore size under certain conditions.

Visscher et al.[122] have found discrepancies between flux ratios of D_2O and osmotic gradients across the small intestine similar to those found in the tissues mentioned above. Their experiments, performed before these principles were appreciated by most investigators, were interpreted in terms of a "fluid circuit" theory,[46, 47] although it is now clear that their data is consistent with the type of hydrodynamic flow found in other cells.

Influence of Bulk Fluid Movement on Solute Movement

Under certain conditions bulk fluid movement will "drag" small solute molecules through the pores in the moving stream. This has been demonstrated quite clearly in the case of toad skin. Ussing and Ander-

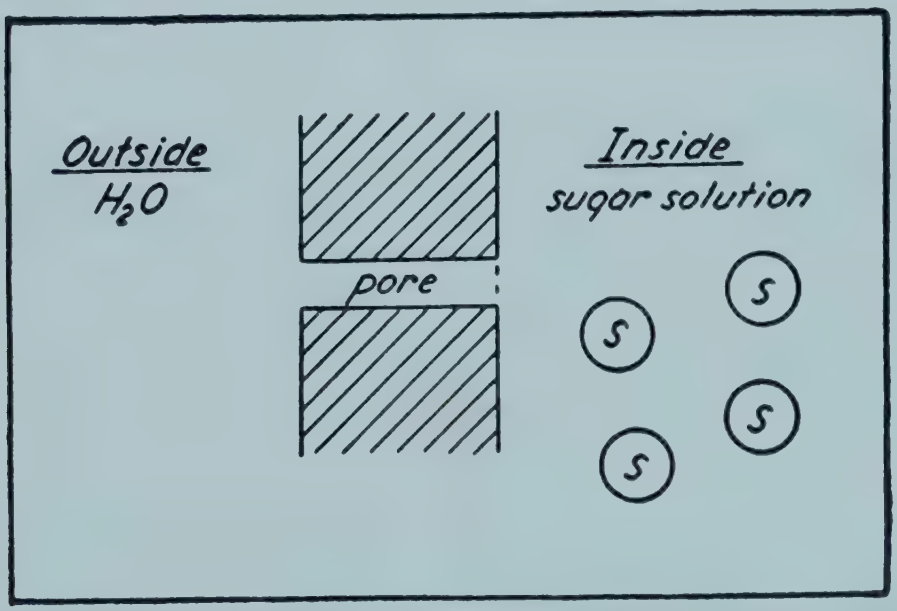


Figure 28. Water-filled pore in cell membrane. (Ussing and Andersen: Abst. 3rd Internat. Cong. Biochem., Brussels.)

sen[118] have shown that vasopressin, which increases osmotic flow of fluid from outside to inside the skin, also increases the rate of passage of thiourea and acetamide in the same direction. Solvent drag might be exerted on ions as well as nonelectrolytes and might cause measurable electrical potentials.

As bulk flow of fluid undoubtedly occurs in the intestine, one might expect to find examples of solvent "drag" on solute molecules during intestinal absorption. The interesting experiment of Fisher[25] may be an example in point. He showed that increased fluid movement across rat intestine *in vitro* was associated with a large increase in the movement of urea, creatinine, and mannitol across the gut wall. A reverse effect is the reduction or abolition of glucose transport in hamster intestine *in vitro* by forced fluid movement in the opposite direction (produced by hydrostatic pressure).[130] One explanation for this is that the glucose molecules were unable to "swim against the current" during massive bulk flow of fluid in the opposite direction.

An effect of bulk flow on an ion movement is illustrated by Cl^- flux measurements of Cooperstein and Brockman.[12] Although the flux rates for Cl^- were very similar in opposite directions across the epithelium of dog colon, there was a distinctly greater flux from blood to lumen during secretion and the opposite during net fluid absorption. These authors interpret their data to indicate solvent "drag" on the Cl^-. Considerably more experimental evidence is necessary to make certain that the previous deductions are correct. It is probably true, however, that bulk fluid movement is of greater importance than was previously believed.

ACTIVE TRANSPORT

Simple passive diffusion probably cannot provide any cell with all its nutrients or maintain the necessary intracellular ionic environment required for survival. All living cells have developed special mechanisms for the translocation of various essential substances across the cell membranes. The special function of the small intestine is the rapid and efficient absorption of the many nutrients required by all the organs of the body. As the rate of diffusion of most water-soluble (and lipid insoluble) substances is rather slow, more efficient mechanisms are required to provide the large amount of foodstuffs necessary for an active organism.

A qualitative comparison of the relative importance of passive diffusion and active transport in intestinal absorption is shown in Table 7. The fluid and electrolyte load of the small intestine of an adult human consists of approximately 7 liters of a solution (approximately isoosmotic) whose predominant ions are sodium and chloride, about 1.5

Table 7. Comparative Importance of Active Transport and Passive Diffusion in Intestinal Absorption

SUBSTANCE	FRACTION ABSORBED BY ACTIVE TRANSPORT	PROBABLE EFFECT OF REMOVAL OF TRANSPORT SYSTEM
NaCl (and H_2O)*	Very large	Death from dehydration in a day or two (without replacement)
Glucose	Large	Malnutrition (plus diarrhea)
Amino acids	Large	Malnutrition
Fats	Large	Steatorrhea, malnutrition
Vitamins	Little or none (except B_{12})	B vitamins: little effect (except B_{12} whose lack results in pernicious anemia) Fat-soluble vitamins: unknown
Nucleic acid derivatives	Little (except uracil and thymine)	Little effect

* It is assumed that salt is actively transported and H_2O follows osmotically.

liters of solution being derived from the diet and 5.5 liters from gastrointestinal secretions. When the active resorptive process of the small intestinal epithelium is seriously impaired by such agents as massive radiation or bacterial infection, the loss of fluid and electrolytes may be so severe as to cause death by dehydration in a day or two unless replacement therapy is quickly instituted.

The efficiency of diffusion in the absorption of carbohydrates, amino acids, and fats is presumed to be poor although little specific data is available for such an estimate. It is known that sugars not actively transported, such as xylose and arabinose, are poorly absorbed and can cause diarrhea if given to human subjects in doses of 25 to 50 gm. Diarrhea in such cases is due to fecal loss of water, osmotically associated with the unabsorbed sugar. Cori[15] noted that rats fed arabinose developed diarrhea within two to three hours while animals fed similar amounts of glucose or galactose absorbed the sugars and never developed diarrhea. *In vitro* the small intestine transports sugars 10 to 20 times faster than the rate of diffusion. It may be concluded that diffusion alone can account for the absorption of only a fraction of ingested glucose. Although there is no specific data for amino acids and fats from which a calculation could be made, it is quite probable that without the active transport systems severe nutritional deficiency would result.

The term "active transport" has often been used to designate any process that appeared to be inconsistent with the laws of simple diffusion. In recent years there has been an attempt to restrict the term to those processes in which a substance moves across a membrane against an electrochemical gradient, and consequently requires energy supplied by cellular metabolism.[97, 115] Furthermore, there must be a direct quantitative

relationship between the energy supplied and the transport work performed. This general definition of active transport as "uphill" movement against an electrochemical gradient requiring expenditure of energy will be used in this discussion. It is obvious that when dealing with charged ions the electrical potential difference across a membrane must be considered.

To indicate the large number of substances absorbed by special transport mechanisms a tentative list is given in Table 8. Excellent evidence is available for the active transport of some substances such as certain sugars and L-amino acids, while only incomplete data are available for Fe^{++} and $SO_4^{=}$. In the case of long-chain fatty acids, diffusion may be involved in entrance into the epithelial cell and some secretory process may be responsible for the exit of the triglyceride particle from the base of the cell. Other compounds will undoubtedly be added to this list in the next few years.

Table 8. ACTIVE TRANSPORT OF SUBSTANCES BY THE INTESTINE AND LOCATION OF MAXIMUM ABSORPTION

SUBSTANCE	LOCATION OF ABSORPTIVE CAPACITY			
	SMALL INTESTINE			COLON
	UPPER*	MID	LOWER	
Absorption				
Sugars (glucose, galactose, etc.)[26, 27]	+ +	+ + +	+ +	0
Neutral amino acids[82, 77, 138]	+ +	+ + +	+ +	0
Basic amino acids[30]	+ +	+ +	+ +	?
Betaine, dimethyglycine, sarcosine[31]	+ +	+ +	+ +	?
Gamma globulin (newborn animals)[9]	+	+ +	+ + +	?
Pyrimidines (thymine and uracil)[105]	+	+	?	?
Triglycerides[5]	+ +	+ +	+	?
Fatty acid absorption and conversion to triglyceride[22, 60]	+ + +	+ +	+	0
Bile salts[70]	0	+	+ + +	
Vitamin B_{12}[4, 14, 95, 113]	0	+	+ + +	0
Na^+[92, 123, 17]	+ + +	+ +	+ + +	+ + +
H^+ (and/or HCO_3^- secretion)[18, 23, 92, 132]	0	+	+ +	+ +
Ca^{++}[102]	+ + +	+ +	+	?
Fe^{++}[24]	+ + +	+ +	+	?
Cl^-[92]	+ + +	+ +	+	0
$SO_4^{=}$[134]	+ +	+	0	?
Secretion				
K^+[18]	0	0	+	+ +
H^+ (and/or HCO_3^- absorption)[18, 23, 92, 132]	+ +	+	0	0
Sr^{++}[124]	0	0	+	?
Cl^- (under special conditions)[114]	+	?	?	?
I^-[93]	0	+	0	0

The references given deal primarily with the effect of location.

* Upper small intestine refers primarily to jejunum, although the duodenum is similar in most cases studied (with the notable exception that the duodenum secretes HCO_3^- and shows little net absorption or secretion of NaCl).

Nonelectrolytes

The simplest case of active transport, from a theoretical point of view, is the movement of an uncharged molecule, such as glucose, across a membrane against a concentration gradient. In this case, the electrical potential differences across the membrane need not be considered. Figure 29 shows the absorption of glucose across isolated small intestine in the hamster. Concentration gradients of over a hundredfold can easily be demonstrated in this preparation. In fact, the maximum concentrating power of the small intestine has never been determined, as the concentration of glucose on the mucosal side often falls to levels indistinguishable from zero. Anaerobically the transport is completely abolished.

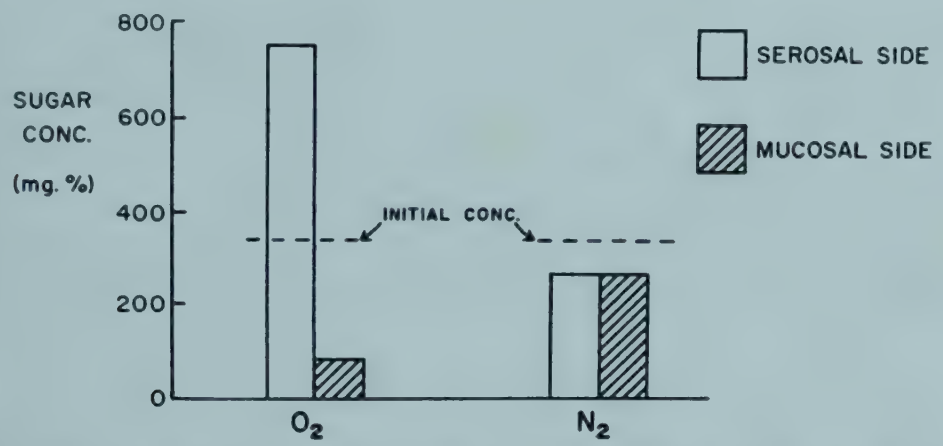


Figure 29. Active transport of glucose by hamster intestine *in vitro* and its inhibition anaerobically. Everted sacs of hamster intestine incubated with initially the same concentration of glucose on both sides of the gut wall, for 1 hour at 37°C. (Wilson and Wiseman: J. Physiol., *123*, 1954.)

Although the adult intestine requires aerobic pathways of metabolism to provide energy for transport processes, the fetal intestine can function satisfactorily anaerobically if glucose is provided for glycolysis. Intestine from fetal or newborn rabbits can transport sugars and amino acids anaerobically.[133] Figure 30 shows that the capacity to transport histidine anaerobically is largely lost during the first week of life.

It has not been possible, thus far, to determine the energy required for a single transport process in the small intestine. The minimal energy required from a thermodynamic point of view for glucose transport *in vitro* may be calculated to be roughly 1 per cent of the total energy produced by the cell. The efficiency of the process, however, is not known. In the one cell in which energetics have been studied (*E. coli*), one mole of ATP is apparently required for the transport of one mole of sugar,[63] an exceedingly inefficient process viewed from the thermodynamic standpoint.

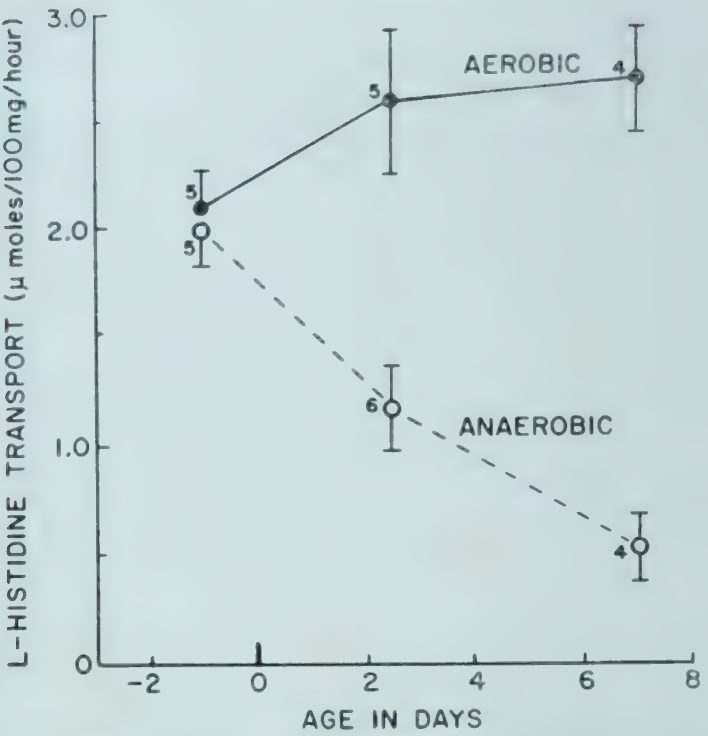


Figure 30. Aerobic and anaerobic transport of L-histidine by rabbit intestine. Everted sacs of rabbit intestine incubated with 5 mM. L-histidine solution on both sides of the wall for 1 hour at 37°C. (Wilson and Lin: Am. J. Physiol., *199,* 1960.)

Although movement against a concentration gradient is the most important criterion for active transport, there are other phenomena expected in such a system. Some of these are:

a. Inhibition of transport with inhibition of energy-yielding reactions.
b. Saturation phenomena (K_m).
c. Competitive inhibition with similar compounds that have a common pathway.
d. Inhibition at low temperature.

The absence of transport when energy-yielding reactions are inhibited is the most useful ancillary phenomenon associated with active transport. This helps to distinguish facilitated diffusion from active transport, as in the former case energy from cell metabolism is not required.

Saturation phenomena, when present, suggest some process other than passive diffusion. At low substrate concentrations, however, active transport is directly proportional to concentration, and therefore similar in this respect to passive diffusion. Competitive inhibition, especially between chemically similar compounds, is frequently found in active transport processes. High temperature coefficients are always found in active transport (and in facilitated diffusion) processes although it is also observed in some cases of passive diffusion through biological mem-

branes. The last three characteristics (b, c, and d), although they are invariably present in active transport, cannot be used to distinguish this from other types of membrane permeation.

In recent years the bacteriologist has added important new information on the nature of certain permeation mechanisms (see recent reviews.[10, 80, 99] Gale[29] has shown that certain gram-positive bacteria accumulate a variety of amino acids. Specific inducible permeation mechanisms for citrate were discovered in 1953.[2, 68] If the pathway for the metabolism of a substance is blocked, the permeation mechanism sometimes results in the intracellular accumulation of the substance. Such a situation has been studied by Monod[81] for thiomethylgalactoside, which will accumulate within the cell against enormous concentration gradients. By a single mutation it is possible to lose or gain the permeation mechanism, which suggests that the mechanism may involve the participation of a single protein molecule.

Electrolytes

The experimental demonstration of active transport of an ion is considerably more difficult than for a nonelectrolyte. The electrical potential difference across the membrane is an additional parameter to be considered. Furthermore, the movement of an ion such as sodium is, under normal conditions, either in exchange for another cation or it is accompanied by some anion to preserve electrical neutrality.

In an isolated system it is possible to simplify greatly the study of such complex processes. For example, solutions containing only a few ions at known concentrations can be added to the tissue. In addition, hydrostatic and osmotic pressures may be controlled, thus minimizing such complicating factors as solvent drag. In order to simplify the electrical parameters of the experiment, Ussing and Zerahn[119] short-circuited frog skin and compared unidirectional and net fluxes of ions with the electrical current across the tissue. Such experiments have been performed on guinea pig ileum by Ussing and Andersen[118] and on toad colon by Cooperstein and Hogben.[13]

An elegant experiment by Cooperstein and Hogben is given in Table 9. In the short-circuited toad colon the chloride flux was the same in both directions, indicating only passive movement. In contrast, the sodium flux from mucosal to serosal sides was seven times that in the reverse direction, indicating active transport of this ion. Furthermore, the close quantitative correspondence between ion movement and current generated indicates that sodium transport is the major source of current in this tissue.

Ussing[115] has derived a useful equation to help distinguish between active transport and passive diffusion of ions. It describes conditions

Table 9. Simultaneous Measurement of Na^+ and Cl^- Fluxes at Zero Potential Difference Across Frog Large Intestine in Vitro

	M ⟶ S	S ⟶ M	NET
Na^+ flux	1.26	0.58	3.68
Cl^- flux	1.45	1.40	0.05
Current			4.0

M ⟶ S indicates mucosal to serosal flux; S ⟶ M indicates serosal to mucosal flux. Current and flux expressed as μEq. cm.^{2} hr. All current is in the direction M ⟶ S. Modified from Cooperstein and Hogben: J. Gen. Physiol., *42*:461, 1959.

for passive diffusion of an ion across a membrane:

$$\frac{M_{in}}{M_{out}} = \frac{a_o}{a_i} e^{zFE/RT}$$

where M_{in} = flux from outside to inside; M_{out} = flux from inside to outside; a_o and a_i are the chemical activities on the outside and inside; z, the number of charges; F, the Faraday number; R, the gas constant; T, the absolute temperature; E, the potential difference across the membrane. This equation has been used by a number of investigators and an example of its use will be given in Chapter 4.

Many other types of experiments have been performed both *in vivo* and *in vitro* which strongly suggest active transport of a number of ions by the small intestine. However, in our present state of ignorance concerning the influence of factors such as diffusible and nondiffusible ions, osmotic and hydrostatic fluid movement, etc., the most satisfactory proof of active transport must be derived from isolated tissues bathed with simple solutions, and with control of electrical parameters.

Mechanisms of Transport

Trapping Reaction. One of the simplest methods of increasing the rate of movement of a substance across a membrane is to reduce its concentration on the opposite side of that membrane by converting it into a second substance. The latter is commonly accomplished by converting the compound into a nondiffusible form. An example of the trapping reaction is the conversion of CO_2 within the cell to carbohydrate or the conversion of a compound into a phosphorylated derivative. The uptake of many small molecules into cells is carried out in a similar manner.

An example of this type might be found in the intestinal absorption of nucleosides. Many nucleosides diffuse into the epithelial cells of the small intestine where they are converted into the corresponding free base[136] and presumably ribose (or deoxyribose) 1-phosphate. The nucleoside-phosphorylase reaction produces one product, ribose-1-phosphate.

which is nondiffusible and presumably would be metabolized by the cell. The reduced concentration of nucleoside within the cell presumably facilitates further entrance of the substance from the intestinal lumen. It must be admitted that this example has not been explored in detail but it illustrates a potential mechanism of intestinal absorption.

Differential Permeability. Under certain conditions the metabolic alteration of one substance into another can be used to provide a driving force for an increased rate of diffusion of a substance across a cellular membrane such as the intestinal epithelium. Let us consider the case of substance A which diffuses from the lumen of the intestine into the epithelial cell where it is converted into compound B, another small, diffusible molecule. One might suppose that compound B would then diffuse out of the cell in both directions. If, on the other hand, the cell membrane at the basal pole of the cell were much more permeable than the brush border end of the cell, compound B would move preferentially across the serosal border. Differential permeability of two membranes of a cell was discovered by Leaf[73] to account for the preferential movement of lactic acid from the epithelial cells of the toad bladder to the serosal side. By direct experimentation Leaf was able to demonstrate that the serosal border of the cell was 14 times as permeable as the cell membrane on the opposite pole of the cell. Although such measurements have not been made in the small intestine it is of interest to see whether this hypothesis might explain some otherwise anomalous data. In Table 10 are shown a number of cases in which a compound enters the epithelial cell from the lumen and is converted to another chemical compound, which then leaves the cell preferentially on the serosal side. In the first five examples given in Table 10 the metabolite produced within the cell could not be actively transported from the mucosal to serosal side against a concentration gradient. As these compounds accumulate on the serosal side only when produced within the cell, the differential permeability of

Table 10. Possible Examples of Transport Through "Differential Permeability"

Compound entering epithelial cell A	Metabolic product leaving epithelial cell B	Observed concentration ratio of B $\frac{\text{serosal concn.}}{\text{mucosal concn.}}$	
Glucose	Lactate	2-10	(129, 137)
Glucose	Pyruvate	1-2	(131)
Fructose	Lactate	—	(66)
Thyroxin analogues	Glucuronide derivative	2-14	(35)
Amino acids	Keto acids	2-4	(125)
Fructose	Glucose	5-10	(135)
Glutamate	Alanine	2-5	(139)
Aspartate	Alanine	—	(139)

References are given in parentheses.

the membranes at the two poles of the cell might explain their asymmetrical distribution. In the last three examples in the table the product within the cell (glucose or alanine) is a compound for which the cell possesses an active transporting process capable of transfer from lumen to blood against a concentration gradient. Recent experiments,[64, 75] however, indicate that the transport process is restricted to the cell membrane facing the intestinal lumen. A substance such as glucose within the cell could not be affected by this membrane-transport system unless it leaked back into the lumen from which it could then be transported. Although this possibility cannot be excluded in the experiments under consideration, differential permeability would seem a reasonable explanation for the preferential movement of intracellular glucose or alanine across the serosal border of the cell. Similar preferential movement of a variety of amino acids of endogenous origin is seen when an everted sac of rat or hamster intestine is incubated in solutions free of amino acids.

Membrane Carrier. One of the most attractive theories accounting for membrane transport is the membrane carrier hypothesis. This hypothesis states that a substance approaches the outer surface of the cell membrane and reacts with a cell wall component. The substrate-carrier complex then moves across a permeability barrier and releases the compound on the inner surface of the membrane. A large number of speculations have been made concerning the type of chemical bonds possibly involved in the substrate-carrier complex and the type of movement the complex might undergo to move across the permeability barrier.

One of the few speculations about a specific chemical reaction that is supported by experimental data is the phosphatidic acid hypothesis for sodium ion transport recently proposed by Hokin and Hokin.[44] These authors have amassed an imposing array of data which support their view that sodium ion reacts with phosphatidic acid at one side of the membrane to produce its sodium salt which in turn moves through a lipid area of membrane to the opposite border of the membrane where the ion is released into the aqueous medium. Two specific enzymes are involved in the breakdown and resynthesis of the carrier molecule (Figure 31).

FACILITATED DIFFUSION

The term "facilitated diffusion" was coined by Danielli[19] to describe a carrier-mediated transport phenomenon in which the rate of attainment of diffusion equilibrium is greatly accelerated although no direct expenditure of energy is required. The fundamental distinction between this process and active transport is the lack of movement against an electrochemical gradient in the former. Facilitated diffusion, however, has some characteristics similar to those of active transport, such as satura-

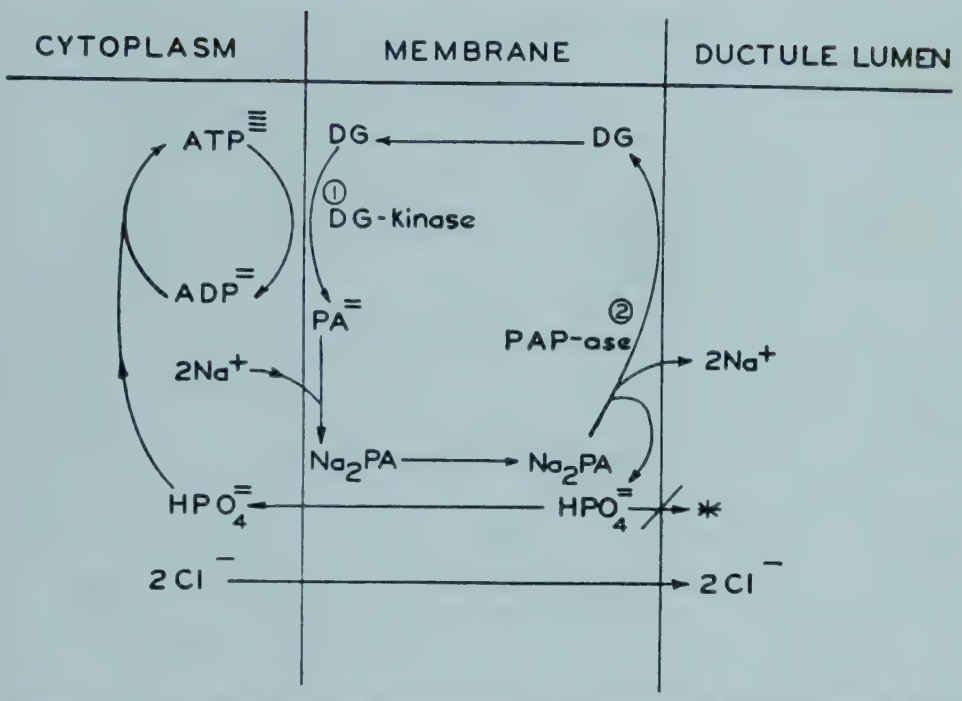


Figure 31. A scheme for the participation of phosphatidic acid as carrier for the active transport of sodium ions across the membrane of the salt-secreting cell. ATP, adenosine triphosphate; DG, diglyceride; DG-kinase, diglyceride kinase; PA, phosphatidic acid; PAP-ase, phosphatidic acid phosphatase. (Hokin and Hokin: J. Gen. Physiol., *44*, 1960.)

tion phenomenon, competitive inhibition by related compounds, and a high Q_{10}. In two carefully studied examples in the erythrocyte (glycerol and glucose permeation), the rate of movement across the cell membrane by diffusion is stimulated a hundred- or a thousandfold by the presence of this special carrier-transport mechanism. Present knowledge of this process stems largely from observations by Jacobs that erythrocytes of certain species possess a special process for glycerol permeability[51] inhibited by traces of copper[54, 57, 58] and competitively inhibited by ethylene glycol.[56] Other workers on this glycerol system[6, 7, 74, 112] have suggested certain chemical groups for the active site.[111] These observations were extended to sugar permeability in the human erythrocyte by LeFevre[74, 75] and others[126, 127] and at present this system is the most carefully studied example of facilitated diffusion.

Although there are undoubtedly a number of examples of this phenomenon in the small intestine, only one has been explored with this specific possibility in mind. Recently Salomon, Allums, and Smith[100] have offered evidence that the intestinal absorption of D-xylose is not through simple passive diffusion but through some type of carrier-mediated process. Their most convincing experiments are of two types. The first established the fact that glucose inhibits xylose absorption, an effect

not consistent with simple diffusion. The second experiment was a study of the effect of glucose on a previously established diffusion equilibrium of xylose across the intestinal wall. In the latter experiment glucose added to the mucosal side of an everted sac of hamster intestine previously equilibrated with xylose resulted in the movement of xylose in the opposite direction from glucose. This transient "counter flow" of xylose apparently occurred against a concentration gradient. Exchange of xylose on one side of a membrane for glucose on the other has been observed in the erythrocyte.[91, 98] Similar exchange phenomena are known to occur in the ascites tumor cell[83] and renal tubule.[85] There is as yet too little data to decide whether xylose has a very weak affinity for the active sugar transport system or whether there is a second distinct transport system shared by xylose and glucose.

Recent studies of Crane, Miller, and Bihler[18] suggest that there is a facilitated diffusion type of mechanism in the intestine available for the sugar 6-deoxy-1,5-anhydro-D-glucitol. Although the compound is not actively transported across the intestine or accumulated within the epithelial cells, its entrance into the epithelial cell is inhibited by phlorizin and stimulated by sodium ions. Such behavior, which cannot be accounted for by simple diffusion, may be explained by some type of carrier-mediated transport system, perhaps similar to that described above for xylose.

There are membrane transport systems for ions with some of the properties discussed in this section on facilitated diffusion. One such system was studied in muscle by Levi and Ussing[78] and labeled "exchange diffusion." Cooperstein and Hogben[33] have found this type of phenomena for Cl^- in the isolated frog large intestine.

PINOCYTOSIS

Phylogenetically the most primitive mechanism for ingestion of food is the process of engulfing particles or dissolved materials by a process of vesiculation. This phenomenon is prominent not only in amebas but in many higher animals.[148] In the embryonic life of mammals ontogeny recapitulates phylogeny with respect to this primitive absorption mechanism.

It has been recognized for many years that cells of the gastrointestinal tract in some animals are frankly phagocytic and digestion of food can be followed within the food vacuoles. Certain flatworms, such as planaria, are especially clear examples of this type.[92, 79, 128] Such phagocytosis is associated with intracellular digestion of large molecules. It is believed that in evolution this intracellular digestion preceded extracellular digestion.[146] Many workers have presumed that the higher animals, whose digestive processes are apparently exclusively extracellular, have com-

pletely lost the capacity to take up substances by vesiculation. In the past few years it has become clear that the intestinal cells of the mammal do in fact possess the ability to absorb certain substances by pinocytosis.

Particularly beautiful photomicrographs for the demonstration of pinocytosis have been published by Clark[9] who has studied protein and particulate absorption by suckling mice and rats. Direct continuity between surface membrane and pinocytotic vesicle is shown in Figure 32. These dramatic morphological changes were stimulated by particulate matter or proteins fed to suckling animals during the first 18 days of life, after which time they completely disappeared. It has been known for 35 years or more that large amounts of intact protein were absorbed by newborn animals of a number of species and, furthermore, that material resembling colostrum could be found in large vesicles in the epithelial cells.[110] The importance of these findings was appreciated only in recent years when the relation of this phenomenon to passive immunity was recognized.

Another recent observation is that of Barrnett[3] who found that duodenal cells of the rat engulfed insoluble dye particles by a process morphologically identical to true pinocytosis (Figure 33). Sanders and

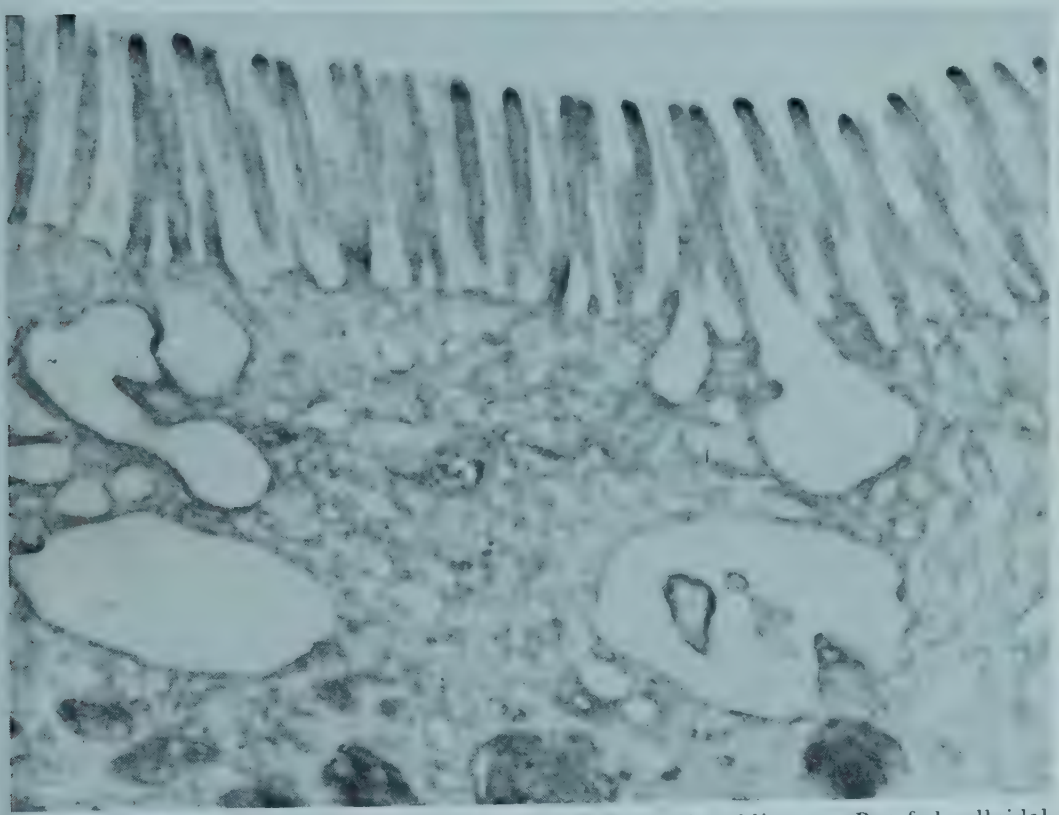


Figure 32. Pinocytosis by intestinal epithelial cell of suckling rat. Rat fed colloidal gold by mouth and the tissue removed after 1 hour. X 22,000. (Clark: J. Biophys. & Biochem. Cytol., *5*, 1959.)

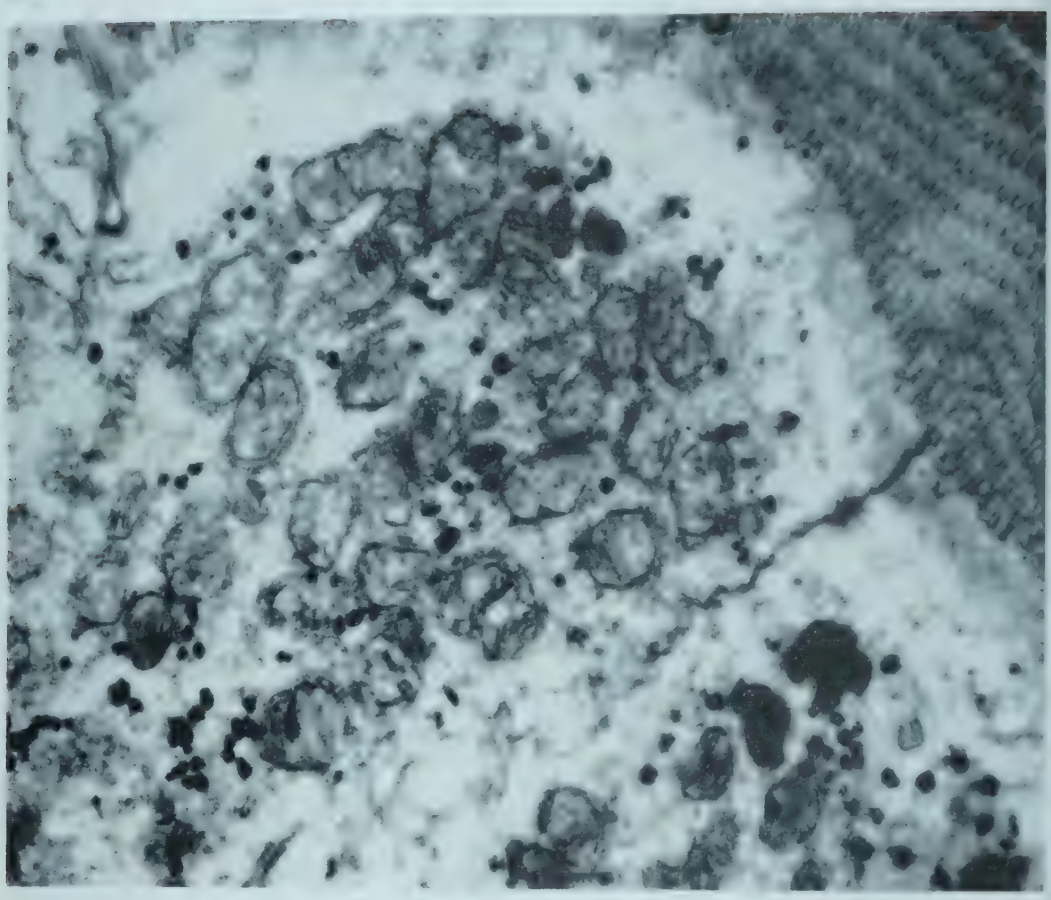


Figure 33. Absorption of insoluble dye particles by rat duodenum. Approximately X 20,000. (Electronmicrograph kindly provided by Dr. R. Barrnett.)

Ashworth[101] have demonstrated particulate absorption in a most unequivocal fashion. They fed latex spheres of about 1000 to 2000 Å in diameter to mice and demonstrated these particles within vesicles in the jejunal epithelium. Furthermore, these spheres were discharged into the lymphatic vessels and later found in many tissues of the body, especially liver and kidney. Palay and Karlin[85] have presented evidence that triglycerides are absorbed by pinocytosis. Their electron microscope pictures show lipid droplets between the microvilli and occasionally at the base of the microvilli. The droplets are next seen in the apical cytoplasm of the cell enclosed in endoplasmic reticulum. The small droplets appear to coalesce in the supranuclear region of the cell and then are extruded from the cell at the level of the nucleus. Photomicrographs of this process will be found in the chapter on fat absorption.

There is a remarkable degree of specificity in some of the absorptive processes believed to depend on pinocytosis. If most lipid is absorbed by pinocytosis (which at the present time is uncertain), this process must have considerable capacity to discriminate between lipid-soluble and

water-soluble constituents. Furthermore, certain lipid-soluble materials such as hydrocarbons or certain steroids are absorbed very slowly. Specificity is also apparent in the protein-absorbing mechanism of the suckling mouse and rat. Homologous antibodies are absorbed considerably faster than certain heterologous antibodies[32, 34] and, furthermore, there is competition between gamma globulins of different species for absorption. If one assumes that these antibodies are indeed absorbed by the pinocytotic process, then certain binding sites must be postulated. Direct evidence for binding sites has been provided in the case of pinocytosis in the ameba (see Holter[45]).

As with other transport processes, the pinocytotic phenomenon seems to show predilection for one portion of the intestine. Clark has shown that morphological changes associated with protein and particulate absorption are most marked in the lower segments of the small intestine.

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CHAPTER 4

Sugars

The understanding of carbohydrate digestion and absorption at any given time in history has always been more advanced than that of other dietary constituents. The study of carbohydrates has been facilitated by the fact that sugars are small, water-soluble molecules which can be estimated readily by specific analytical methods. Furthermore, considerable information has been available on their chemistry and metabolism. Thus, it was possible as early as 1879[154] to write the following description of digestion and absorption of sugars: "There is reason to believe that most of the dextrose into which all carbohydrates are converted in digestion is absorbed by the veins."

In the succeeding eighty years progress occurred spasmodically, with advances usually related to some new experimental method or new idea. At the present time we seem to be on the verge of making a further advance in the elucidation of the biochemical mechanism of absorption and the factors in its control.

CARBOHYDRATES PRESENT IN THE DIET

Many human diets contain as much as 500 gm. of carbohydrate per day, although, of course, this figure varies over a wide range. The two main types of dietary carbohydrates are polysaccharide and disaccharide, with a great variety of additional glycosides and free sugars present in

smaller amounts. The epithelial cells of the small intestine are virtually impermeable to the large molecular weight polysaccharides. It has been reported that minute quantities of intact starch grains may be absorbed from the intestine and appear in the blood and urine[82, 169] but if it occurs it is quantitatively insignificant. Neither cellulose nor inulin is absorbed, as such, by the intestine. Although they cannot be hydrolyzed by mammalian enzymes, these compounds may be degraded by bacteria in the stomach of ruminants or in the colon[87] of other animals. There is no convincing evidence that appreciable quantities of starch or any intermediate in its hydrolysis by amylase (except maltose) are absorbed. Even in the case of maltose comparatively little absorption occurs because of the extremely active maltase and the relatively slow rate of absorption of the disaccharide. There is, however, evidence for limited absorption of disaccharides, particularly of lactose, of which hydrolysis in the intestinal lumen is quite slow.

Watkins[177] has studied lactose absorption in human subjects by estimating the sugar in the urine after ingestion of various quantities of sugar. Urinary excretion is a satisfactory measure of absorption if, as in the case of lactose, practically no hydrolysis or metabolism occurs within the body and it is rapidly excreted in the urine. He found that 10 gm. was the largest amount of lactose which could be fed to normal men without the appearance of appreciable amounts in the urine. Similar results were obtained with women in the intermenstrual period, while 20 gm. or more could be tolerated by women at the time of menstruation without lactosuria. Whether this represents changes in lactase content of the intestine or changes in absorptive capacity is not known.

With *in vitro* methods it is possible to compare the rate of starch hydrolysis with the rate of absorption of split products. When starch was placed on the mucosal side of hamster intestine *in vitro* (Figure 31) extensive hydrolysis occurred resulting in the appearance of glucose, maltose, and many polysaccharide fragments of different molecular weight (slow running spots on a paper chromatogram). In this experiment over 95 per cent of the sugar appearing on the serosal side was glucose with only a trace of maltose and no oligosaccharides.

Many monosaccharides, particularly of the hexose and pentose series, are absorbed, and a wide variety of different sugars have been identified in human urine.[184] Of the common natural sugars only glucose and galactose are absorbed by an active transport process, while the rest are absorbed either by some other type of carrier-mediated transport[125] or by passive diffusion. It should be emphasized that animals are capable of absorbing considerable amounts of sugars by simple diffusion.

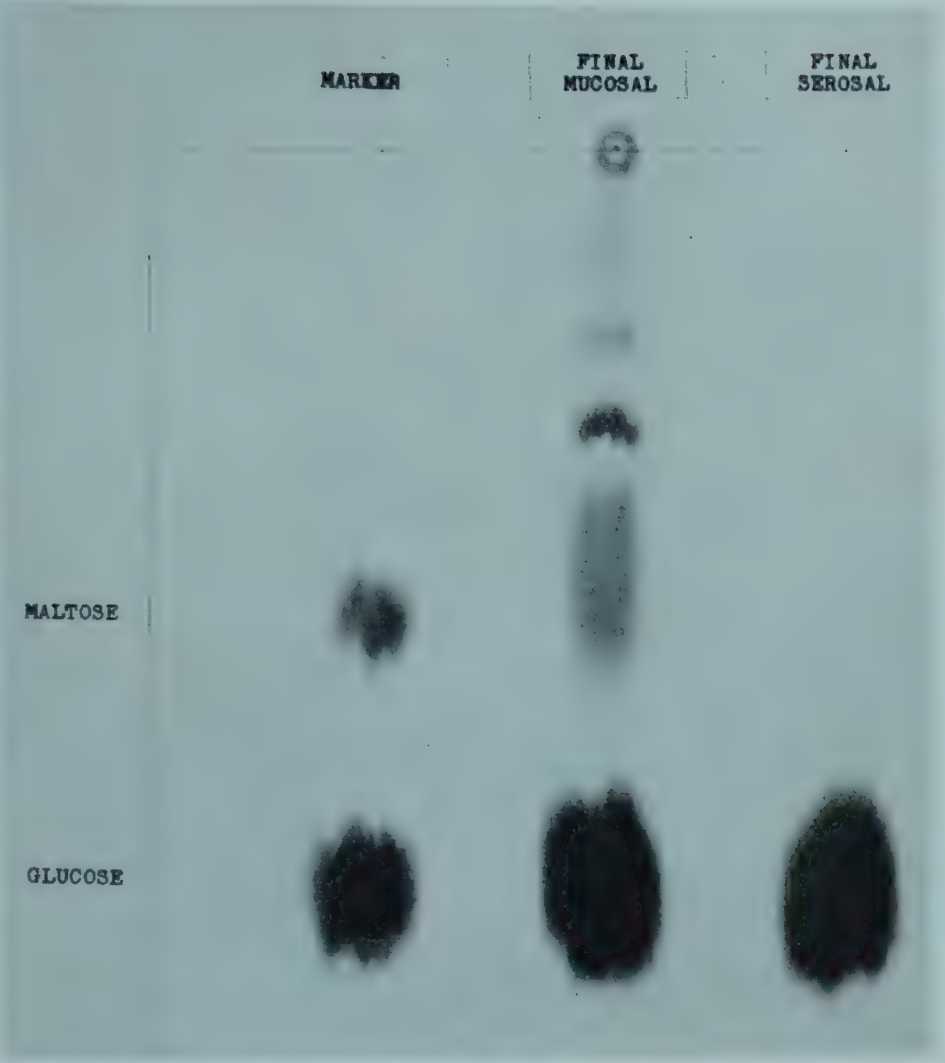

Figure 34. Paper chromatogram showing hydrolysis of starch by hamster intestine. An everted sac of jejunum containing 2.0 ml. of sugar-free solution was incubated in 5 ml. of a 1 per cent solution of starch for 1 hour. Note that of all the starch hydrolysis products only glucose crossed the gut wall. (Wilson and Vincent: J. Biol. Chem., *216,* 1955.)

HYDROLYTIC ENZYMES OF THE SMALL INTESTINE

A brief statement about the carbohydrate-splitting enzymes of the small intestine is appropriate. As starch is the predominant carbohydrate in the diets of most animals, the hydrolytic enzyme, amylase, assumes considerable importance. Salivary amylase splits the 1,4-glycosidic link in a random fashion and produces maltose, maltotriose, and, in the case of amylopectin, small branched oligosaccharides.[148] Pancreatic amylase, although similar in many respects, produces, in addition to these hydrolysis products, considerable quantities of free glucose[121, 183] (Figure 35). These enzymes are highly active and cause extensive hydrolysis of starch in

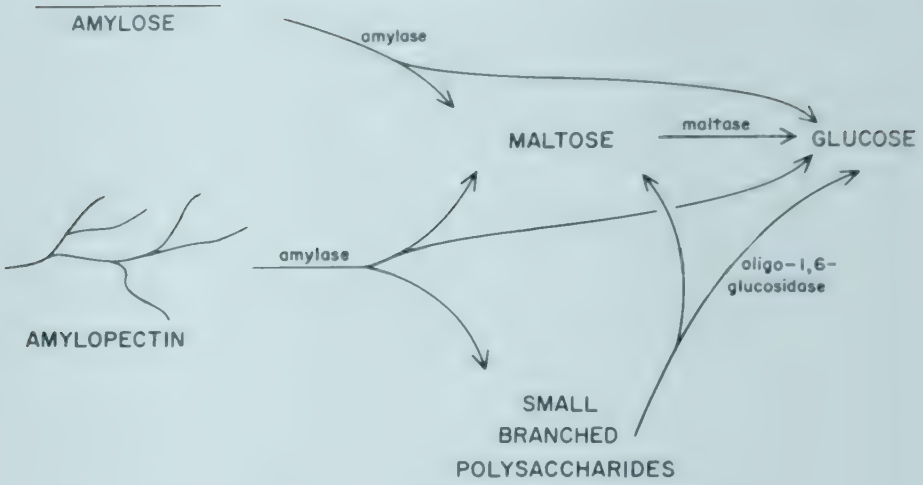

Figure 35. Hydrolysis of starch in the intestinal tract.

the upper portion of the small intestine. Considerable salivary amylase escapes inactivation in the stomach because it is protected by a bolus of food and as much as half of the starch may be hydrolyzed before it reaches the duodenum. In addition, amylase is present in the epithelial cells of the small intestine. The branch points of the amylopectin are cleaved by an enzyme of the small intestine, oligo-1, 6-α-glucosidase (distinct from the debranching enzyme of the liver.)[104] Finally, intestinal maltase splits the remaining disaccharide maltose to produce glucose.

The hydrolysis of the three common disaccharides, maltose, sucrose, and lactose, is carried out by enzymes of the small intestine. Lactase, or β-galactosidase, is highly active in mammals at the time of birth, and decreases in activity during the first few months of life to the low levels found in the adult.[7, 176] Maltase and invertase activity, on the other hand, rise during the first few weeks of life from very low levels in the newborn animal to the adult level.[7] To what extent these changes may be due to "adaptation" to ingested sugars is not clear.

A few authors still hold the view that maltase, invertase, and lactase are secreted by some unidentified cells of the small intestine into the *succus entericus* where they react with the disaccharides in the lumen. However, Cajori,[18] studying Thiry-Vella loops in dogs, showed that sucrose and lactose disappear from the lumen much more rapidly than would be accounted for on the basis of the hydrolytic enzymes found in the *succus entericus*. He further showed[19] that, while insignificant amounts of lactase were present in the secretions, large amounts were present in the intestinal mucosa, enough to hydrolyze completely the lactose disappearing in the loop experiments. He concluded[19] that lactose was not appreciably affected by the enzyme in the intestinal secretions but was hydrolyzed intracellularly or by "direct contact with

the mucosa." The enzyme activity in the intestinal secretions is probably entirely from desquamated epithelial cells.[108]

With regard to the location of hydrolytic enzymes, there is histochemical evidence for localization of phosphatase and lactase in the brush border of the cell.[57, 86] Furthermore, phosphatase[149] and nucleotidase[196] appear to be imbedded in the outer surface of the brush border with their enzymatically active sites facing the lumen (outside the permeability barrier of the cell membrane). Miller and Crane,[122] on the other hand, believe that invertase and other hydrolytic enzymes are in the area of the brush border but within the permeability barrier.

A deficiency of one or more of these disaccharide-splitting enzymes occasionally occurs in humans and may be responsible for clinically important symptoms. The cause of certain cases of diarrhea and weight loss in young children has been traced to the inability of the gut to split a certain disaccharide with consequent excretion of the unabsorbed sugar with an osmotic equivalent of fluid. Holzel, Schwarz and Sutcliffe[84] described the first case of infant malnutrition from defective lactose absorption. Weijers et. al.[179] studied a number of cases with deficiencies of maltase, invertase, or lactase. The diagnosis was made indirectly with a variety of ingenious methods including oral tolerance tests ([a] disaccharide, [b] monosaccharide, [c] disaccharide plus disaccharase) and fecal and blood analyses of short-chain fatty acids.[178]

When the disaccharide is not hydrolyzed in the small intestine it enters the colon where hydrolysis and fermentation by bacteria occurs. A patient lacking invertase passes large amounts of fatty acids in the stool following sucrose ingestion, but not on a sucrose-free diet. It appears that enzyme deficiency persists for many years (if not for life) and that there is a definite familial relationship, with more than one in a family often affected.

ABSORPTION OF SUGARS INTO PORTAL BLOOD

Von Mering in 1877[174] was the first to consider carefully the relative importance of the portal vein and lymphatics as routes of absorption. He found that during sugar absorption the concentration of glucose in the portal vein was the same as or greater than that in the thoracic duct. Furthermore, he presented evidence that the sugars passed directly into the blood vascular system, as during absorption the concentration of glucose in the blood leaving the gut (portal blood) was higher than that entering it (arterial blood). Although he came to the definite conclusion that absorption of sugars was via the portal vein, it remained for others to provide more convincing proof of his hypothesis.[110, 76, 155, 100, 98, 97, 5] Some glucose is always present in the lymph and Munk and Rosen-

stein[124] recovered 0.5 gm. of sugar from the lymph of a human subject after a meal of 100 gm. of carbohydrate. A particularly clear analysis of available data was made by Hendrix and Sweet,[76] who concluded that the walls of the blood capillaries and lymph vessels were freely permeable to glucose and that the absorption of glucose by the portal veins was due to the very rapid flow rate of blood compared to that of lymph. In the rat, for example, compare a splanchnic blood flow of 400 ml./hr.[144] with a thoracic duct flow of 0.5 ml./hr.[161]

An elegant modern method for the study of this problem was devised by Shoemaker et al.[159] and involves the introduction of catheters into the portal vein, hepatic vein, and splenic artery. This method permits not only sampling of portal blood in an unanesthetized dog but also a reasonably accurate measure of blood flow so that quantitative studies of absorption may be made. An example of such an experiment is given in Figure 36.[160] The concentration in the portal vein was always greater than that in the artery and at one point (ten minutes after glucose ingestion) it was double that in the artery. A small percentage of the glucose appears in portal blood as lactic acid. Calculated from the blood flow and the venous-arterial difference, over 90 per cent of the administered glucose could be accounted for as glucose in the portal blood.

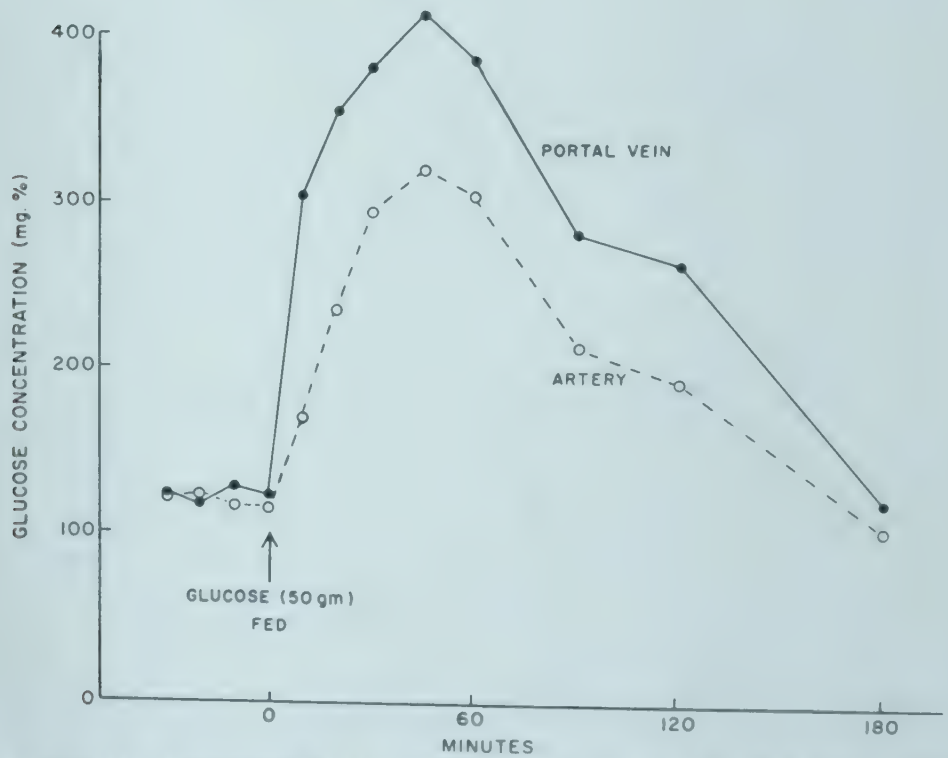

Figure 36. Appearance of glucose in portal vein blood during glucose absorption in an unanesthetized dog. (Shoemaker et al.: unpublished observations.)

A few studies have been performed in human subjects in which portal vein blood has been sampled indirectly. Patients with severely restricted blood flow through the liver (cirrhosis) develop collateral venous drainage from the intestine which sometimes includes a superficial vein on the surface of the abdominal wall. Such anastomotic veins contain "portal blood" which can be sampled before and after feeding a test substance to the individual. This unique clinical opportunity of sampling "portal blood" was apparently first appreciated by Sherlock and Walche[158] who, in studying a patient with cirrhosis, found a rise in glucose concentration in an abdominal anastomotic vein after glucose feeding. More extensive studies with a variety of test substances have been carried out by others.[13, 11]

CHEMICAL FORM IN WHICH GLUCOSE APPEARS IN PORTAL BLOOD

The transformation of glucose into other substances during intestinal absorption has been frequently suggested in the older literature. When adequate experimental methods became available it was found that most of the glucose lost from the lumen of the intestine appeared in the portal vein as reducing sugar (presumably identical with glucose). Hestrin-Lerner and Shapiro[78-80] recently questioned this view with the observation that radioactive glucose placed in the lumen of the intestine *in vitro* or *in vivo* was converted to an unidentified compound which appeared in the blood. On the basis of *in vitro* experiments Wilson and Wiseman[188-190, 197, 198] suggested that this unidentified compound might be lactic acid. They had found that a considerable amount of glucose entering the epithelial cell was converted to lactic acid, which appeared in high concentrations in the serosal solution. Subsequently Kiyasu and Chaikoff[97] found that when the portal vein of anesthetized rats was cannulated only a minor portion of the absorbed glucose appeared as lactic acid. The present position appears to be that in the dog[5, 168] and the rat[98] glucose appears, in the portal blood, predominately as free glucose with relatively small amounts of lactate. No other compounds have been found. Galactose has not been studied extensively *in vivo* but this sugar probably appears exclusively as galactose in the portal vein. Fructose is more complicated, producing a mixture of fructose, glucose, and lactic acid, the proportions depending on the species of animal. In the rat up to 50 per cent of the fructose may appear in the portal blood as lactic acid while in the guinea pig most fructose appears as glucose.[97]

LOCATION IN THE INTESTINE OF SUGAR ABSORPTION

The upper portion of the small intestine has a greater capacity to absorb glucose than the lower, while the stomach and colon absorb little if any sugar. Reid,[148] for example, showed in 1900 that the upper ileum absorbed more glucose than the lower ileum and the colon absorbed very little. The duodenum is apparently quite active in sugar absorption. Abbott et al.[1] found that human duodenum had the capacity to absorb more than 10 gm. of glucose per hour. Fisher and Parsons,[65] from their measurements of the mucosal surface area[66] and the absorption rate, calculated that glucose absorption (which included utilization as well as transport) per unit of mucosal area was about three times as rapid in the upper jejunum as the lower ileum. Other studies indicate that, at least *in vitro*, the lower jejunum transports somewhat more sugar per weight of tissue than segments just above or below.[67, 42]

The intestine has a considerably greater capacity for sugar absorption than is normally required of it. In a 300 gm. rat a dose of 100 mg. of glucose is completely absorbed by the first half of the small intestine.[145] Borgström et al.[16] recently studied the site of glucose absorption in human subjects. They fed a test meal containing glucose (55 gm.) and lactose (20 gm.) as carbohydrate, as well as polyethyleneglycol (a nonabsorbed indicator substance), and sampled the intestinal contents at various levels of the small intestine through a small tube swallowed by the subject. By comparing the concentration of glucose with that of polyethyleneglycol they could calculate the percentage absorption at the various levels. Absorption began in the duodenum and was complete in the proximal 100 cm. of the jejunum (the total length of human small intestine is approximately 300 cm.).

SELECTIVE ABSORPTION OF SUGARS IN VITRO

During the latter half of the nineteenth century a number of physiologists, notably Hamburger and Höber, showed that the laws of diffusion could explain certain observations on intestinal absorption of electrolytes and nonelectrolytes. It came as somewhat of a surprise, therefore, when Hédon[73] and Negano[123] found that the hexoses, glucose and galactose, were absorbed from the intestine more rapidly than the pentoses, xylose and arabinose. The significance of these observations was not fully appreciated, however, until Cori in 1925 carefully investigated the problem of sugar absorption with unanesthetized rats[31] and found that there was a striking specificity in the absorbing mechanism (Figure 37). Mannose, which differed from glucose only in the configuration of the hydroxyl group at carbon 2, was absorbed at about one fifth

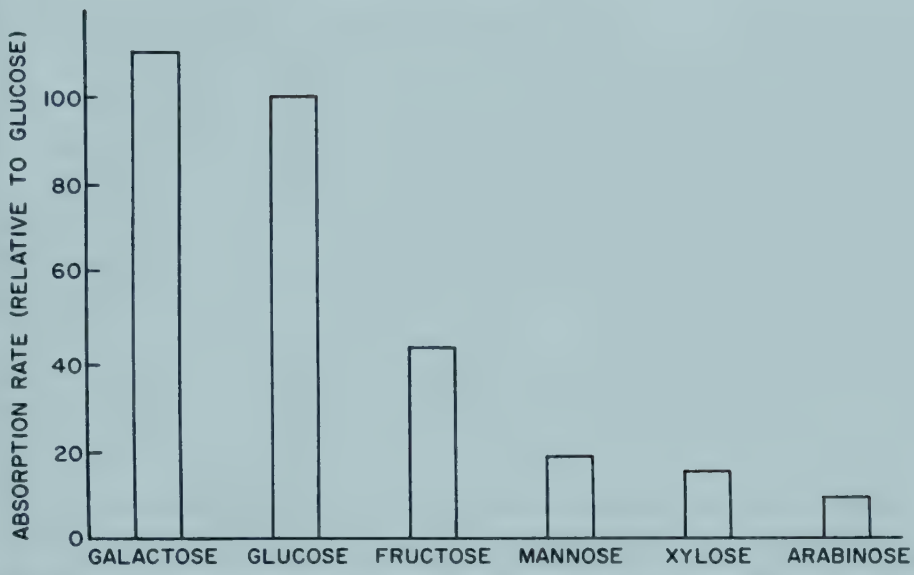

Figure 37. Selective sugar absorption by unanesthetized rats. Sugar was fed by stomach tube and the absorption rate was determined as the disappearance of sugar from the gastrointestinal tract in a given period of time. (Drawn from the data of Cori: J. Biol. Chem., *66*:691, 1925.)

the rate of glucose. Cori's data were interpreted in terms of "selective permeability" (the term "active transport" had not yet been coined). These results were confirmed in the case of the rat with a variety of different experimental methods and extended to other animal species

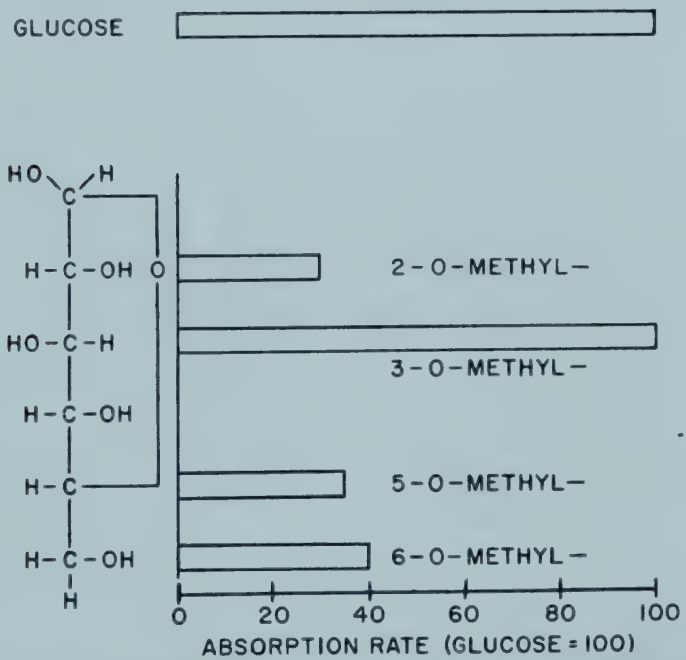

Figure 38. The absorption rate of different -0-methyl derivatives of glucose in the rat. The rate of disappearance of the sugar from a loop of intestine of an anesthetized rat was measured. (Drawn from the data of Csáky: Ztschr. physiol. Chem., *277*:47, 1943.)

(Table 11). Only in the case of the cat and one type of fish did the absorption rate of a pentose approach that of glucose.

The first study of specificity with synthetic sugars was that of Csáky who showed that the rate of absorption of 3-0-methylglucose was similar to that of glucose, while the corresponding 2-0-methyl, 5-0-methyl, and 6-0-methyl derivatives were absorbed at much slower rates[45] (Figure 38). In a further study he found that 1-0-methylfructose and 3-0-methylfructose were slowly absorbed by the rat but not against a concentration gradient.[51]

The selective nature of the absorption process is further illustrated by the effect of sugar concentration on the absorption rate of different sugars. In general, sugars absorbed by passive diffusion show a linear relationship between concentration and absorption while sugars actively transported show saturation phenomena. The early sugar feeding experiments were complicated by high concentrations in the lumen and

Table 11. Selective Absorption of Sugars by Different Animals

ANIMAL	AUTHOR	RATE OF SUGAR ABSORPTION (GLUCOSE TAKEN AS 100)					
		GALACTOSE	GLUCOSE	FRUCTOSE	MANNOSE	XYLOSE	ARABINOSE
Rat	Cori[31]	110	100	43	19	15	9
	Wilbrandt & Laszt[186]	115	100	44	37	30	29
	Westenbrink[181]	108	100	42	15	13	2
	Sols & Ponz[163]	106	100	40		24	15
	Vidal-Sivilla[172]	100	100	30	7	13	6
	Hele[75]	112	100	52	25	26	
Dog	Nagano[125]	>*	>	>	>	>	>
Cat	Hewitt[81]	90	100	35			
Cat (ileum)	Davidson & Garry[56]	125	100	50		94	
Rabbit	Hédon[73]	82	100				60
Hamster	Wilson & Vincent[195]	88	100	16	12	28	10
Man	Groen[71]	122	100	67			
	McCance & Madders[119]					(3.6)†	(2.3)†
Pigeon	Westenbrink[181]	115	100	55	33	37	16
Frog	Westenbrink & Gratama[182]	118	100	54	64	12	
	Csáky & Fernald[48]		100			22	
	Minibeck[123]	96	100	38	38	44	
Fish	Cordier & Worbe[30]	89	100	77		49	9
	Cordier & Chanel[26]	102	100	45		78	80
	Cordier et al.[27]	99	100	65		43	57

* Only qualitative comparisons can be made from the data, which show a descending order of absorption rate with galactose the most rapid and arabinose the slowest.
† The reference substance here is not glucose but rhamnose which is taken as 1.

uncertain gastric emptying time, which resulted in divergent opinions in the literature. Cori found a constant absorption rate when 25 per cent, 50 per cent, and 80 per cent solutions were administered to unanesthetized rats.[31] Although this was confirmed by some,[35, 168, 166] other workers[116, 142, 2, 9, 61, 171, 146, 62] found a distinct increase in absorption rate resulted from increasing the luminal concentration of glucose. A reasonable explanation for the latter observations was proposed by Donhoffer[60] on the basis of experiments with phlorizin. He found that with increasing concentrations of glucose, phlorizin always inhibited the absorption of the same quantity of sugar, leaving increasing amounts of sugar unaffected by the glycoside. He concluded that phlorizin completely inhibited the active absorption of glucose but had no effect on diffusion (which increased with increasing luminal concentrations). If his inferences were correct, considerable diffusion of glucose occurred at high luminal concentrations, Bárány and Sperber[9] came to a similar conclusion from a kinetic analysis of their data on glucose absorption in the rabbit.

Somewhat more reproducible results were obtained by studying absorption from isolated tied loops of intestine in anesthetized animals. Verzár[168] showed that while the absorption rate of glucose and galactose was relatively constant over a certain range of concentrations, the rate for sorbose, mannose, and xylose increased in a linear relation to concentration (see Figure 23, p. 42, for data on sorbose). This suggested simple diffusion as the mechanism of absorption for the last three compounds and some other process for glucose and galactose.

INHIBITION OF SUGAR ABSORPTION

An important milestone in the history of intestinal absorption was the suggestion by Wilbrandt and Laszt[186] that the mechanism of sugar absorption involved a phosphorylation reaction. This hypothesis was based on the observation that iodoacetate, which had been shown to inhibit glycolysis, inhibited the absorption of glucose and galactose but not that of mannose, xylose, or arabinose (Table 12). Believing that iodoacetate was specific for phosphorylation reactions they proposed that sugars were phosphorylated during transport through the intestinal epithelium. In the same year, Lundsgaard proposed phosphorylation as the mechanism for glucose resorption from the renal tubules.[114]

This theory was important because it was the first suggestion of a specific biochemical reaction to account for a cellular transport mechanism. The general phosphorylation-dephosphorylation hypothesis gained considerable support from a variety of experiments and the question seemed entirely settled for many years. The current status of this hypothesis will be discussed later (p. 99).

Table 12. Effect of Iodoacetate on Selective Sugar Absorption

Sugar	Absorption rate (glucose taken as 100)	
	Control	Iodoacetate
Galactose	115	53
Glucose	100	33
Fructose	44	37
Mannose	33	25
Xylose	30	31
Arabinose	29	29

Sugar absorption measured in tied loops of intestine of anesthetized rats. Taken from Wilbrandt and Laszt: Biochem. Ztschr., *259*:398, 1933.

The plant glycoside, phlorizin (Figure 39), has played a central role in the study of glucose absorption as, in low concentrations, it appears to specifically inhibit sugar absorption by the intestine and renal tubule.[141, 156, 175] In 1922 Nakazawa[127] showed that this poison inhibited glucose absorption without affecting absorption of amino acids, fat, or salts. Furthermore, phlorizin inhibits the absorption of only glucose and galactose, not xylose, arabinose, and sorbose.[180, 60, 17] An example of its effect on active sugar transport is shown in Table 13. Following the enunciation of the phosphorylation theory, attempts were made by the proponents of the theory to demonstrate that this material, like iodoacetate, inhibited phosphorylation reactions. In these studies, large concentrations (10^{-3} M. or greater) of phlorizin were always required to produce inhibition of phosphorylation.[113] Much lower concentrations (10^{-4} to 10^{-5} M.) will produce drastic effects on sugar absorption.[15, 148, 85] More recent biochemical studies with phlorizin by Shapiro[157] and Lotspeich[111] indicate that concentrations of 10^{-4} M. will affect the citric acid cycle. Lotspeich has recently discussed current views and concluded that this glycoside exerts its effect by reacting with some permeation mechanism located probably on the outer surface of cells.[111] In confirmation of this general view is a study by Newey, Parsons, and Smyth[129] who present evidence that phlorizin inhibits the mechanism responsible for the passage of glucose across the luminal border of the epithelial cell.

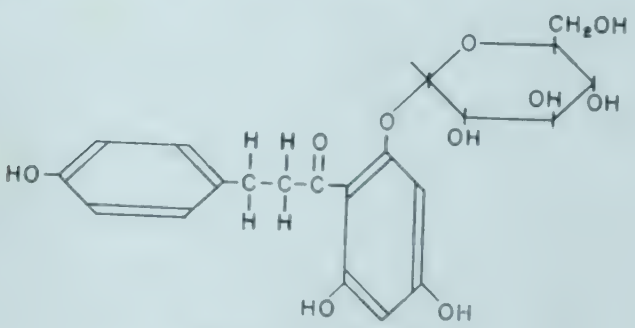

Figure 39. Phlorizin.

Table 13. Accumulation of Sugars by Strips of Hamster Intestine

TEST SUGAR	CONC. IN MEDIUM	CONDITIONS	TISSUE CONCENTRATION / MEDIUM CONCENTRATION
6-Deoxyglucose	0.25 mM.	Control	17.2
		Nitrogen	0.7
		Phlorizin (1 mM.)	0.7
D-Xylose	1.0 mM.	Control	0.4
		Nitrogen	0.3

Strips of intestine incubated for 40 min. at 37°C. with sugar. Taken from Crane and Mandelstam: Biochim. et. biophys. acta, *45*:460, 1960.

Inhibition of energy-yielding reactions of the epithelial cells uniformly leads to loss of active glucose absorption. Anaerobic conditions,[25, 55, 197] cyanide,[55] dinitrophenol,[16, 55, 150] malonate,[55] and fluoroacetate[55] all inhibit glucose absorption by *in vitro* preparations of small intestine. The many other compounds known to inhibit glucose absorption include atabrine,[17] arsenite,[137] uranyl ion,[136] molybdate,[138] copper,[139] and azide.[55] The review of Cordier[24] may be consulted for a discussion of many other factors affecting absorption.

ABSORPTION AGAINST A CONCENTRATION GRADIENT

An important advance in our understanding of sugar absorption was the demonstration that glucose could be absorbed from the intestine against a concentration gradient. This was first demonstrated by Bárány and Sperber,[9] who were studying changes in concentration of glucose solution placed in the intestine in the anesthetized rabbit (Figure 40). It was well known that, when dilute glucose solutions were instilled into the intestine, both sugar and water were rapidly and completely absorbed. Bárány and Sperber devised the ingenious experiment of adding the poorly absorbed salt, sodium sulfate, to the sugar solution, thus preventing the complete absorption of fluid. Under these conditions they were able to demonstrate that the glucose concentration in the lumen fell to levels much below that in the blood. An experiment is given in Figure 40 in which the sugar concentration in the intestinal loop fell to zero while the blood concentration remained at 200 mg./100 ml. Although metabolism of the glucose without transport to the portal blood was not excluded, this experiment suggested that the epithelial cells could transport glucose against considerable concentration gradients. Campbell and Davson[20] later performed a theoretically more convincing experiment with the nonmetabolizable sugar 3-0-methylglucose. This compound was absorbed from the lumen of the cat intestine despite the high blood level of the compound maintained by intravenous injection. These observations

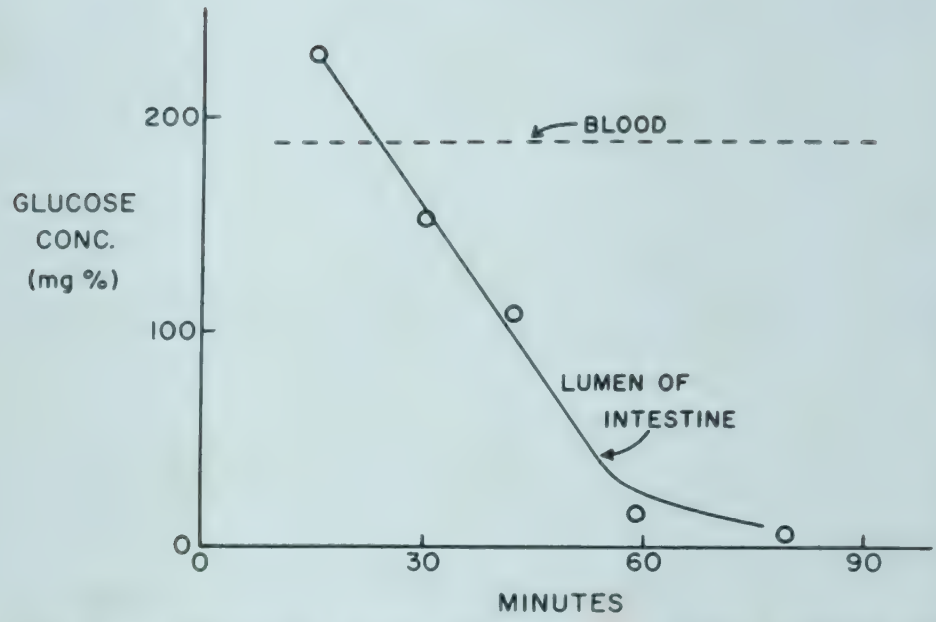

Figure 40. Absorption of glucose from the lumen of rabbit intestine against a concentration gradient. A glucose solution containing sodium sulfate was placed in a loop of intestine of rabbit anesthetized with ether. (Drawn from the data of Bárány and Sperber: Skandinav. Arch. Physiol., *81*:290, 1939.)

were subsequently confirmed and extended by both *in vivo*[5] and *in vitro* methods.[64, 197]

RECENT STUDIES WITH IN VITRO METHODS

During the past decade there has been a renewed interest in intestinal absorption, due largely to the introduction of satisfactory *in vitro* methods. In 1919 Fisher and Parsons[64] described the first well-oxygenated, *in vitro* method for the study of the small intestine. By circulating oxygenated bicarbonate-saline separately on the two sides of isolated rat intestine they were able to maintain the activity of the epithelial cells for an hour or more. The experimental design was to place the same solution on both sides of the intestinal wall and observe changes in fluid volume and sugar concentration. In one experiment the initial glucose concentration on the mucosal side of 416 mg./100 ml. fell during incubation to 228 mg./100 ml. while the concentration on the opposite side rose from 459 mg./100 ml. to 502 mg./100 ml. There was a net movement of glucose across the full thickness of this *in vitro* gut preparation against a concentration gradient of more than twofold. This was an unequivocal demonstration of net glucose transport against a concentration gradient by the small intestine.

Kinetic Studies

In vitro methods lend themselves especially well to studies where rigid control of experimental conditions is required. Fisher and Parsons[67] found that glucose and galactose absorption by isolated rat intestine conformed closely to the Michaelis-Menten kinetics (Figure 41). They emphatically state that these kinetics do not necessarily indicate that an enzyme is involved and point out (quite properly) that "absorption on to a component of the cell membrane" would produce similar kinetics if it were the rate-limiting step. Furthermore, the assumptions involved in the derivation of the Michaelis-Menten equation are correct in only a limited number of purified enzyme systems and are unlikely to be entirely valid in the complex system of sugar absorption, involving as it does an unknown number of steps of unknown type. It is useful, nevertheless, to estimate the apparent affinity constant (K_t) for a number of compounds to assess the relative affinity of the system for these compounds. K_t is used in this book rather than K_m to emphasize the many assumptions made in dealing with such a complex system as transport in an intact tissue preparation. A few apparent K_t values taken from *in vitro* experiments are given in Table 14. There are, as one might expect, some variations from animal to animal and from one tissue preparation to another. The inhibitory effect of one sugar on another, however, is in remarkably good agreement with that predicted from the K_t values when the experiments are all performed on the same tissue under similar conditions.

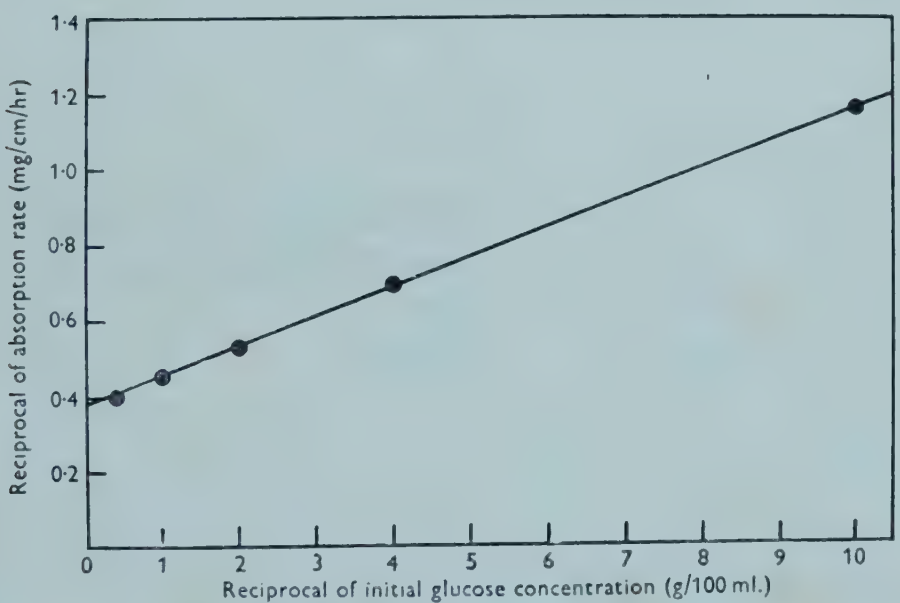

Figure 41. Linear relation between the reciprocal of the glucose concentration in the lumen and the reciprocal of the rate of glucose absorption by rat intestine *in vitro*. (Fisher and Parsons: J. Physiol., *119*, 1953.)

Table 14. The Affinity (K_t) of Sugars for Intestinal Transport

Sugars	K_t values (mM.) in the following animals:			
	Rat[67, 68]	Guinea pig[147]	Hamster 44, 89 "test tube" method	Hamster[37] tissue accumulation method
Glucose	9	7	2.5*	1.5
Galactose	35		12*	2.2
3-0-methylglucose			10*	
6-deoxyglucose			0.55*	
1-deoxyglucose			1.0	7.4

* Experiments carried out at 30°C.; all other experiments at 37°C.

Unidirectional Movement of Different Sugars

The rate of diffusion of a substance across a simple membrane should be the same in both directions. Figure 42 shows the results obtained with the sac method for a number of sugars.[195] The rate of movement in the two directions was similar in the case of fructose and xylose. With glucose and galactose, on the other hand, the rate of movement from mucosal to serosal sides was much greater than it was in the reverse direction. Presumably much of the glucose which leaks from serosal side into the epithelial cell or into the mucosal solution is transported back across the gut. In another type of experiment the flux rate of radioactive glucose from serosal to mucosal sides was measured during net transport in the opposite direction. The ratio of flux rate in the direction of absorption (mucosal to serosal) to the flux rate in the opposite direction (serosal to mucosal) gave a value of about 20/1.[190]

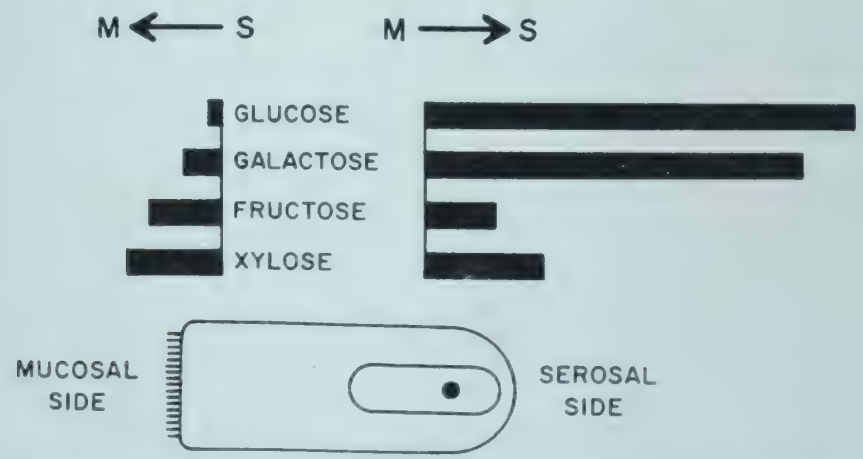

Figure 42. Unidirectional flux rates for different sugars across hamster intestine. Everted sacs of hamster intestine were incubated with a sugar (0.03 M.) on one side or the other and the rate of passage to the other side measured. (Drawn from the data of Wilson and Vincent: J. Biol. Chem., *216*:851, 1955.)

Effect of Temperature

It is well known that the diffusion of substances across a cellophane membrane is affected only very slightly by changes in temperature. It has been erroneously reasoned that diffusion through biological membranes should show the same behavior, and any process strongly inhibited by low temperature must involve an enzymatic reaction. Although it is quite true that studies involving the effect of temperature on a process may sometimes be useful, such studies must be interpreted with caution. Auchinachie, Macleod, and Magee[6] found that while the rate of movement of both glucose and xylose across rabbit intestine *in vitro* was reduced by reducing the temperature from 40°C. to 0°C., the rate of movement of glucose was reduced proportionately greater than that of xylose. Historically this was an important observation, since, in 1930, the concept of "active transport" of glucose and passive diffusion of xylose was not recognized. In the hamster intestine reduction of temperature to 0°C. almost completely stops the movement of either glucose or xylose.[195] A high Q_{10} for glucose absorption would be expected and was experimentally observed by Cordier and his collaborators[29, 28] in a number of fish. It is concluded that the study of the effect of temperature is interesting in a number of regards but high temperature coefficients may be observed for both active and passive movement across cell membranes.

Effect of Ions

Both sodium and potassium ions appear to be necessary for optimal glucose absorption. Ricklis and Quastel[147] have shown that increasing the potassium concentration from 5 mM. to 20 mM. caused a doubling of glucose transport in isolated guinea pig intestine. A much more dramatic effect on sugar absorption was observed when alterations were made in the sodium concentration on the mucosal side of the intestinal wall. When sodium was completely replaced with potassium,[147, 47, 52 54, 43, 23, 151] lithium, magnesium, or mannitol[47, 54] active sugar transport completely ceased. Furthermore, cardiac glycosides which inhibit sodium transport in a variety of cells inhibited glucose absorption.[50, 43] The possibility that sodium transport and glucose absorption are linked together is considered in a later section.

Lactic Acid Production During Glucose Absorption

During experiments on glucose absorption with rat intestine *in vitro,* Wilson and Wiseman[188, 197, 198, 190] found that considerable quantities of lactic acid were produced. Furthermore, the concentration of lactate on the serosal side of the intestinal wall was much higher than that on the mucosal side. In these experiments the pH on the mucosal side fell while that on the serosal side remained constant. These authors

inferred that some of the glucose entering the epithelial cell from the lumen was converted into lactic acid, the hydrogen ion being secreted into the lumen and the lactate appearing preferentially on the serosal side. It was suggested that this was one of the pathways for glucose absorption. The presence of a lactate gradient has been confirmed both *in vitro*[130, 131] and *in vivo*[98] but its quantitative significance in glucose absorption is questionable.

Subsequent studies indicated that *in vivo* this pathway was responsible for only about 10 per cent of the glucose absorbed in the dog[5] and less than 10 per cent in the rat.[98] On the other hand Kiyasu and Chaikoff[97] showed that as much as 50 per cent of the fructose absorbed in the rat appeared in the portal blood as lactic acid. Probably the lactate pathway is quantitatively important only in the case of fructose absorption in certain animals.

Specificity of the Active Transport System

Transport against a concentration gradient had been shown *in vivo*[8, 20, 5] and *in vitro*[65, 68] for a few sugars, but the methods did not lend themselves to the study of milligram quantities of a large number of compounds. The development of the everted sac method by Wilson and Wiseman[197] permitted such a study of intestinal transport of a wide variety of synthetic compounds. To date more than 70 sugars and sugar derivatives have been tested with sacs of hamster intestine.[197, 195, 39, 40, 191, 193, 103]

Figure 13 shows data for the transport of several compounds which may be considered modifications of the glucose molecule at carbon atom 1. One interesting compound is 1-deoxyglucose (1,5-anhydro-D-glucitol) which Crane and Krane[39] found to be transported. Glycosides, which are modifications of the hydroxyl group at carbon 1, are also transported. Most of the glucosides and galactosides studied by Landau, Bernstein, and Wilson[103] were actively transported, even compounds with an aglycone as large as a phenyl group (Table 15). It was concluded from this data that the hydroxyl group at carbon 1 was not essential for transport. In contrast, the hydroxyl at carbon 2 appeared to be essential (Figure 43). The replacement of this hydroxyl group by hydrogen, as in 2-deoxyglucose, produced an inactive compound. Other compounds with modifications at carbon 2 which were not transported included 2-deoxygalactose, 2-O-methylglucose, mannose, glucosamine, and acetylglucosamine. The hydrogen on carbon 2, however, could be replaced without loss of activity, as was suggested by the active transport of 2-C-hydroxymethylglucose.[40]

Additional experiments with other compounds indicated that the hydroxyl groups at carbon 3, 4, and 6 were not essential (Figures 43 and

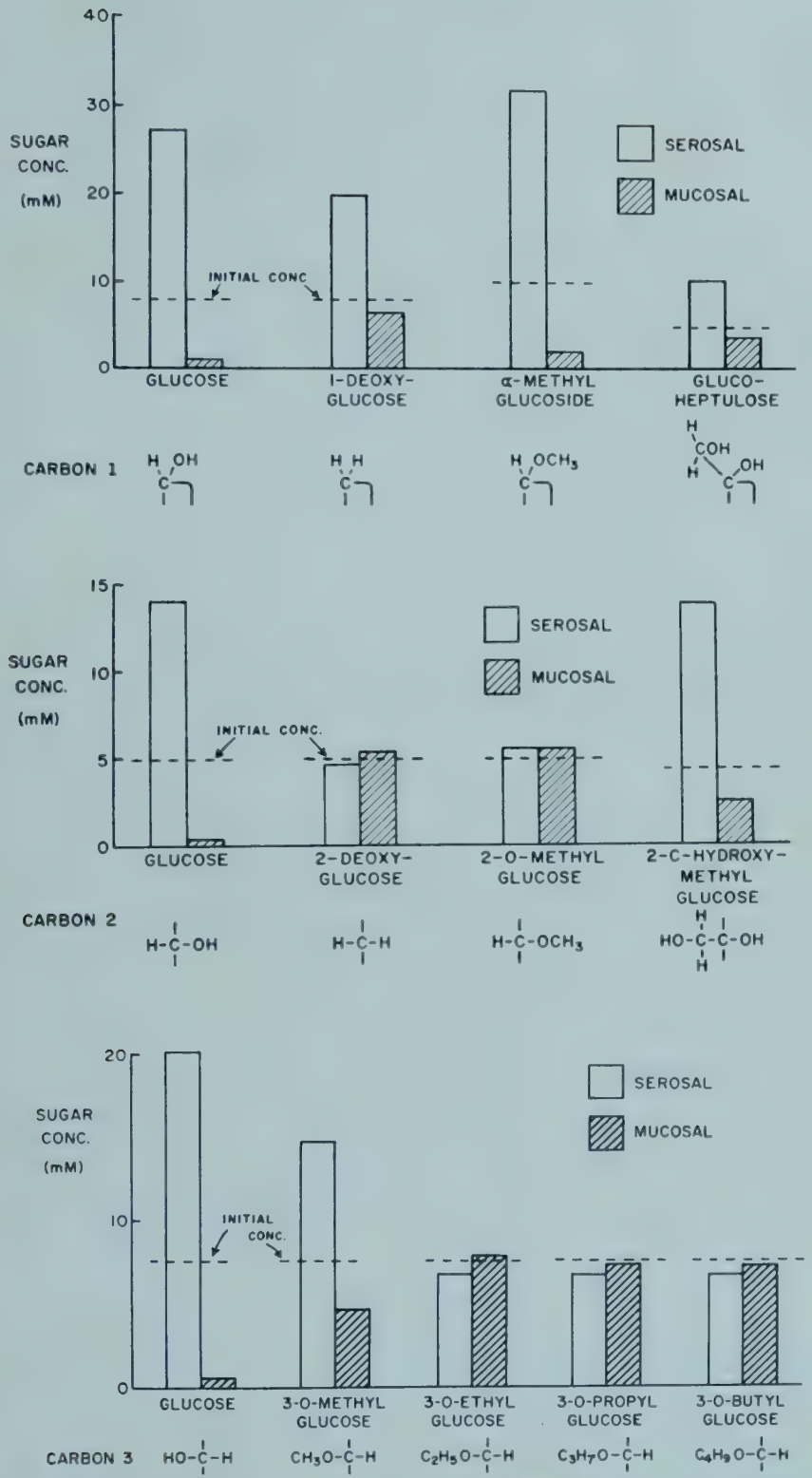

Figure 43. Transport of compounds with modifications at carbon 1, 2 or 3 of the glucose molecule. Figures were constructed from published data for 1-deoxyglucose[39]; glucoheptulose and 2-deoxyglucose[44]; 2-c-hydroxymethylglucose[40]; and others.[193] (Upper and middle panels from Wilson et al.: Fed. Proc., *19*, 1960. Lower panel from Wilson and Landau: Am. J. Physiol., *198*, 1960.)

Table 15. Active Transport of Glycosides by Hamster Intestine

TRANSPORTED	NOT TRANSPORTED (OR VERY SLOWLY)
Glucosides:	Glucosides:
α-methyl	α-butyl
β-methyl	β-isobutyl
α-ethyl	
β-ethyl	
β-isopropyl	
β-butyl	
β-sec butyl	
α-phenyl	
β-phenyl	
β-p-chlorophenyl	
β-hydroquinone	
β-2ethoxyethyl	
β-monobromallyl	
Galactosides:	
α-methyl	
β-methyl	
β-methyl-thio	

Active transport against a concentration gradient by sacs of hamster intestine. Taken from Landau, Bernstein, and Wilson: Am. J. Physiol., in press.

44). Furthermore, none of the pentoses tested were transported. The important features appear to be a D-pyranose ring structure with a hydroxyl group of the glucose configuration at carbon 2. It should be noted that this generalization was based on alterations of single substituents on the glucose molecule. Changes of configuration at more than one carbon atom might give rise to inactive compounds, even though single modifications were tolerated. (E.g., D-gulose, which differs from glucose in the configuration of the hydroxyl groups at carbons 3 and 4, was not transported.) Furthermore, compounds with sufficiently large substituent groups were not transported,[193] probably because of steric effects or effects on the conformation of the sugar (e.g., 3-O-ethyl, 3-O-propyl and 3-O-butyl glucose). Stereospecificity is indicated by the fact that D-glucose is transported but not its L-enantiomorph.[193, 48, 152]

Before any general conclusions on the specificity of the transport system could be derived, it was important to determine whether all transported sugars share a common pathway. The first investigations demonstrated competitive inhibition between glucose and galactose[32, 68] and between 3-O-methylglucose and glucose.[46] Crane[37] then found that 1-deoxyglucose inhibited glucose accumulation by segments of isolated intestine (Table 16). Keston and Tyree failed to observe inhibition with this sugar combination with the sac method.[94] Additional combinations of sugars have recently been tested for mutual competitive inhibition

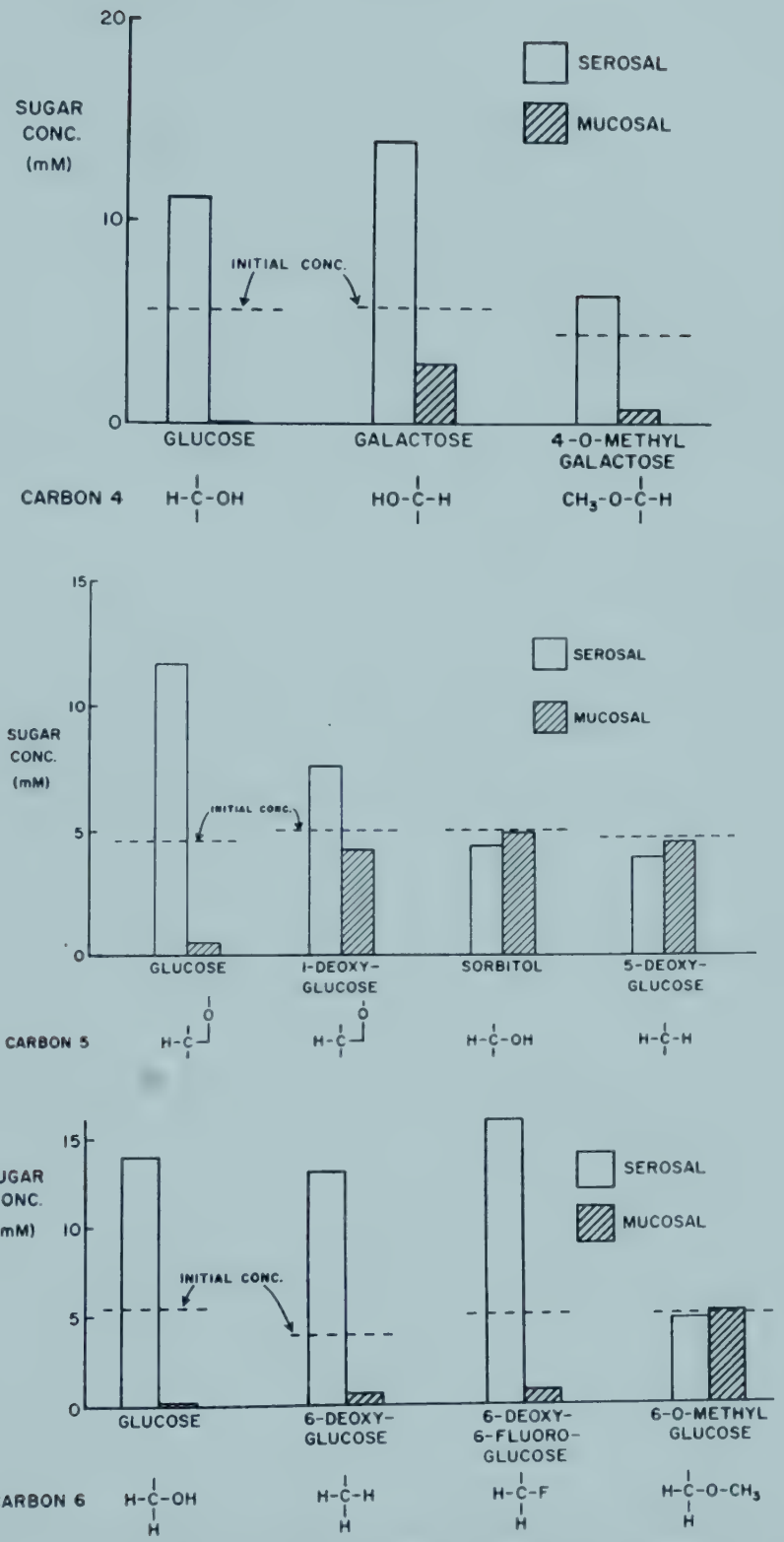

Figure 44. Transport of compounds with modifications at carbon 4, 5, and 6 of the glucose molecule. The figure was constructed from published data for 1-deoxyglucose and 6-deoxyglucose[39]; 5-deoxyglucose[103]; and others.[193] (Top and bottom panels from Wilson and Landau: Am. J. Physiol., *189,* 1960.)

Table 16. Mutual Inhibition Between Glucose and 1-Deoxyglucose*

TEST COMPOUND	COMPOUND ADDED	TISSUE CONC. (mM.)	PER CENT INHIBITION	
			FOUND	CALCULATED†
Glucose (1mM.)	None	2.76		
Glucose (1mM.)	1-deoxyglucose (25mM.)	1.67	44	63
1-(H^3)deoxyglucose (25 mM.)	None	2.4		
1-(H^3)deoxyglucose (25 mM.)	Glucose (1mM.)	1.0	58	61

* More properly designated 1, 5-anhydro D-glucitol.

† Per cent inhibition calculated by taking glucose as $K_t = 1.5$ and 1 deoxyglucose as $K_t = 7.4$ and assuming that the two sugars share a pathway.

Strips of hamster intestine were incubated either with glucose, with 1-deoxyglucose, or with both sugars. Tissue concentrations of the sugars were determined after two-minute incubation. In the tissue exposed to both sugars, each was determined by independent methods (glucose by glucose oxidase and 1 deoxyglucose by radioactivity). Taken from Crane: Biochim. et biophys. acta, *45*:477, 1960.

by the method of Crane and Wilson.[44] A simplified experimental design is presented in Figure 45 in which two time periods were studied with the same segment of intestine, first with 6-deoxyglucose plus inhibitor (glucose), and next with 6-deoxyglucose alone.[88, 89] Glucose inhibited the transport of 6-deoxyglucose and the inhibition was reversible. With glucose as the test sugar, inhibition was observed with both 1-deoxyglucose and α-methylglucoside. In a study of galactose transport, mannose

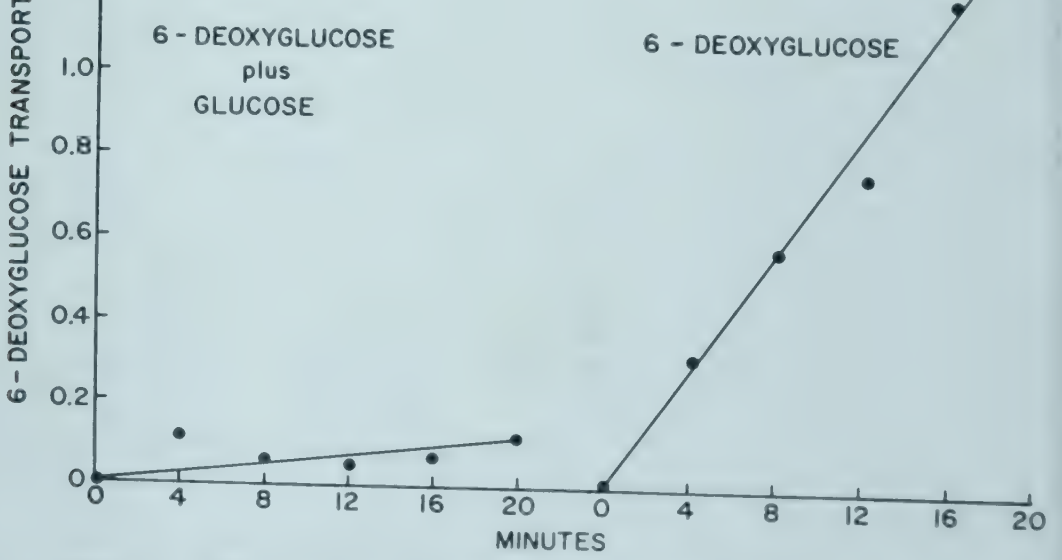

Figure 45. Effect of glucose (12 mM.) on the transport of 6-deoxyglucose (1 mM.). (Wilson et al.: Fed. Proc., *19*, 1960.)

and xylose had no effect while glucose, 6-deoxyglucose, and 3-0-methylglucose inhibited galactose significantly. In every case examined, the presence of one transported sugar inhibited the transport of the second.

These data strongly indicate that a common pathway is involved in sugar transport. This conclusion allows one to delineate the minimal structural requirement for the sugar transport system by hamster intestine from the experiments on specificity (Figure 46).

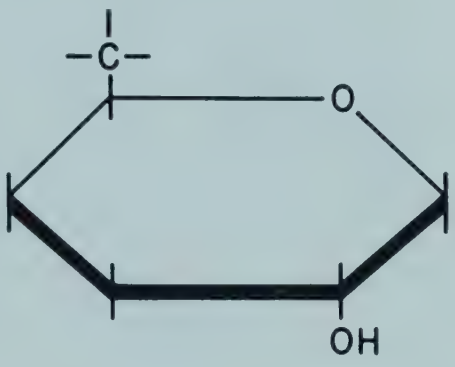

Figure 46. Minimum structural requirements for intestinal transport of sugars. (Wilson et al.: Fed. Proc., *19,* 1960; and Crane: Physiol. Rev., *40,* 1960.)

FRUCTOSE ABSORPTION

It has been known since the work of Cori[31] that the rate of absorption of fructose was between those of the rapidly absorbed sugars, glucose and galactose, and those of the slowly absorbed ones such as mannose and xylose. In 1931 Bollman and Mann[15] showed that intravenously injected fructose could be converted to glucose by the gastrointestinal tract of the dog. A few years later Laszt[105] reported experiments in which fructose was converted to glucose by homogenates of intestinal mucosa and Kjerulf-Jensen[99] reported a rise in blood glucose after fructose feeding to a rabbit. Pillai and Saxena[135] have recently found that a similar interconversion occurs in the intestine of the cockroach.

Particularly convincing experiments on the fructose-to-glucose conversion during absorption were provided by Darlington and Quastel[55] and also by Fridhandler and Quastel,[69] who identified glucose by means of the specific glucose oxidase method. The rate of movement of fructose appeared to be roughly proportional to the luminal concentration while the rate of its conversion to glucose remained relatively constant over a wide range of fructose concentrations (Table 17). When fructose was placed on both sides of sacs of hamster intestine, no transport against a concentration gradient occurred (Figure 47). The concentration of fructose fell on both sides of the intestinal wall and the glucose formed appeared largely on the serosal side.[195]

Another aspect of fructose absorption is its conversion to lactic acid by the intestine in some animal species. Kiyasu and Chaikoff[97]

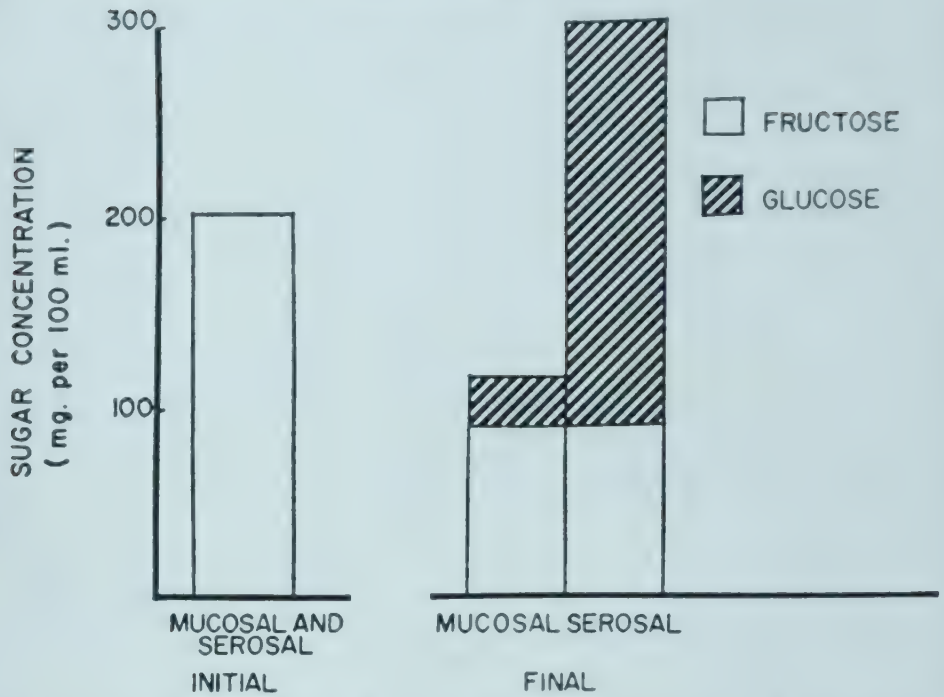

Figure 47. The absorption of fructose by hamster intestine. Fructose (200 mg. per cent) was placed on each side of the intestinal sac. (Wilson and Vincent: J. Biol. Chem., *216,* 1955.)

Table 17. FRUCTOSE ABSORPTION BY ISOLATED GUINEA PIG INTESTINE

FRUCTOSE CONC. ON MUCOSAL SIDE (mM.)	APPEARANCE OF SUGAR ON SEROSAL SIDE	
	FRUCTOSE μMOLES/HR.	GLUCOSE μMOLES/HR.
7	2.1	19
14	5.6	22
28	15	19

Taken from Table VI of Riklis and Quastel: Canad. J. Biochem. & Physiol., *36*:347, 1958.

cannulated a mesenteric vein in the rat and collected venous blood during absorption of radioactive fructose. As much as 50 per cent of the radioactivity in the portal plasma was lactic acid. The conversion of glucose to lactate as one mechanism of sugar absorption was suggested by Wilson and Wiseman[188, 197] on the basis of *in vitro* experiments with rat intestine. There are apparently differences between species in lactate production, as the guinea pig produces very little.[97]

The mechanism of the interconversion of fructose and glucose is apparently similar to that discovered by Hers and Kusaka in liver[77] and is shown in Figure 48. Fructose-1-phosphate, the product of fructokinase, was first isolated by Kjerulf-Jensen from the intestinal mucosa of

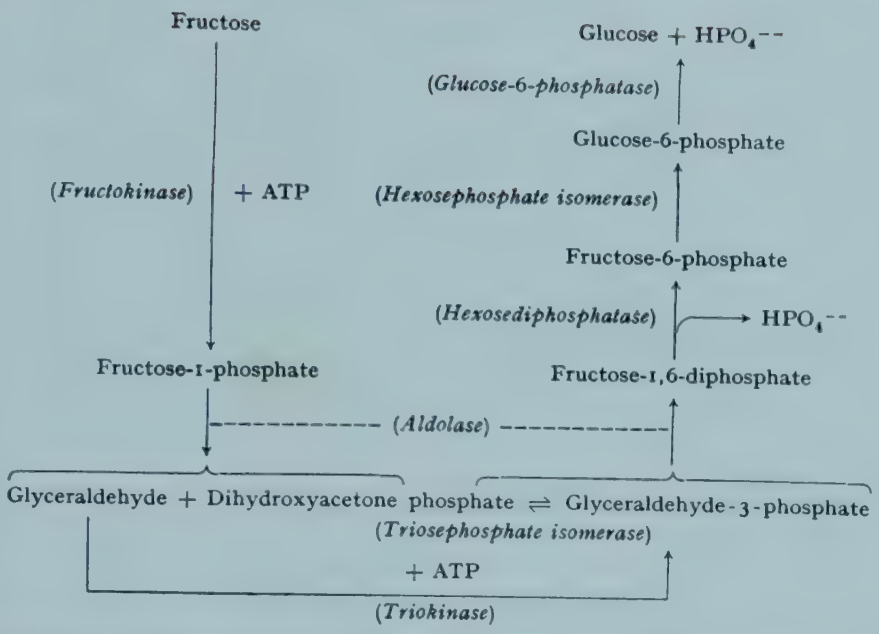

Figure 48. Pathway of conversion of fructose to glucose in liver. (Hers and Kusaka: Biochim. et biophys. acta, *11*, 1953.)

rats and rabbits during fructose absorption. Presumptive evidence for its presence in guinea pig gut has been presented by Ginsburg and Hers.[78] According to the Hers scheme (Figure 48) fructose is converted into two triose phosphates which can be interconverted by an isomerase so that when two triose phosphates are converted to hexose again, carbon 1 of the original fructose will become equally distributed between carbons 1 and 6 of the resulting glucose molecule.

The predicted randomization of the label from fructose-1-C^{14} in its conversion to glucose was found independently in two laboratories.[70, 153] Glucose-6-phosphatase, the final enzyme in the sequence, was found in large amounts in the guinea pig but was absent or found in small amounts in the rat. The lack of this enzyme in the rat is presumed by Ginsburg and Hers[70] to account for the very poor conversion of fructose to glucose in this animal. They make the following inference with regard to the pathway in human intestine: "If this is true, the fact that glucose-6-phosphatase could not be detected in human intestine might indicate that fructose is not converted to glucose in the course of digestive absorption in man."

Fructose, then, is unique among sugars in intestinal absorption. Although by itself it is not actively transported by the small intestine, it may be converted in the intestinal epithelium into two separate metabolic products, glucose and lactic acid, which are discharged into the blood. Figure 49 illustrates these pathways of fructose absorption.

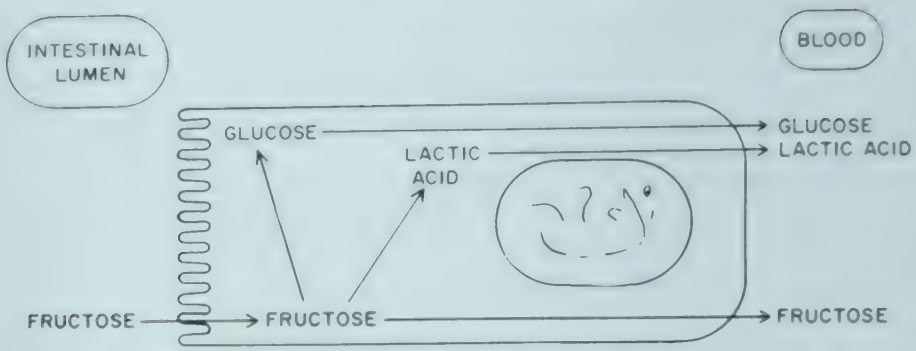

Figure 19. Different pathways of fructose in the epithelial cell of the small intestine.

XYLOSE ABSORPTION

Some extremely interesting observations on D-xylose absorption have recently been reported by Salomon, Allums, and Smith.[152a] These authors observed two phenomona which are not consistent with the view that xylose crosses the intestinal wall by passive diffusion. The first observation was that glucose inhibited the movement of D-xylose from mucosal to serosal sides of guinea pig intestine *in vitro*, when the sugar was moving from a higher to a lower concentration. The fact that D-xylose is not actively transported against a concentration gradient, and that it does not appreciably inhibit galactose transport, led most workers to believe that this pentose has no affinity for the glucose transport system. This new observation, however, suggests that glucose and D-xylose share some carrier system.

The second observation was the induced counterflow of xylose by the addition of glucose. In this experiment everted intestine was incubated with radioactive D-xylose on the mucosal side until sugar equilibrium was reached (90 min.). Glucose was then added to the mucosal side and a transient increase in D-xylose concentration on the mucosal side was observed. This behavior could occur only in the presence of some type of membrane carrier which had affinity for both glucose and xylose.

The explanation for this interesting observation is not yet clear. One possibility is that D-xylose has a slight affinity for the sugar transport system, but such a slight affinity that no measurable transport against a gradient can be observed. If this were the case a compound such as phlorizin with a strong affinity for the carrier should radically inhibit D-xylose movement. Another possibility, of course, is that there is a separate transport system for D-xylose for which glucose happens to have an affinity. Further experiments are required to resolve this question.

LOCALIZATION OF PERMEATION MECHANISMS

A full description of glucose transport across the epithelium of the small intestine requires detailed knowledge of the intracellular localization of the different parts of the transport system and the permeability barriers. Newey, Parsons, and Smyth[129] have shown that phlorizin, at concentrations which do not affect endogenous respiration (less than 10^{-3} M.), inhibits the metabolism of glucose from the mucosal side of the cell as well as net transport, while utilization from the serosal side is unaffected. It was concluded that phlorizin inhibits the mechanism responsible for movement of glucose across the luminal border of the epithelial cell.[129]

Additional information has been gained in the study of sugar accumulation during intestinal transport. Intestinal tissue will accumulate glucose,[67] galactose,[68] 6-deoxyglucose,[42, 41] and others[42] during the transport of these compounds *in vitro*. If the primary transport system were located at the luminal border of the epithelial cell, accumulation of sugar would be expected to occur within the columnar epithelium. If, on the other hand, the primary transport were located at the base of the epithelial cell, accumulation would occur below the cell in the *lamina propria*. McDougal, Little, and Crane[120] recently showed that

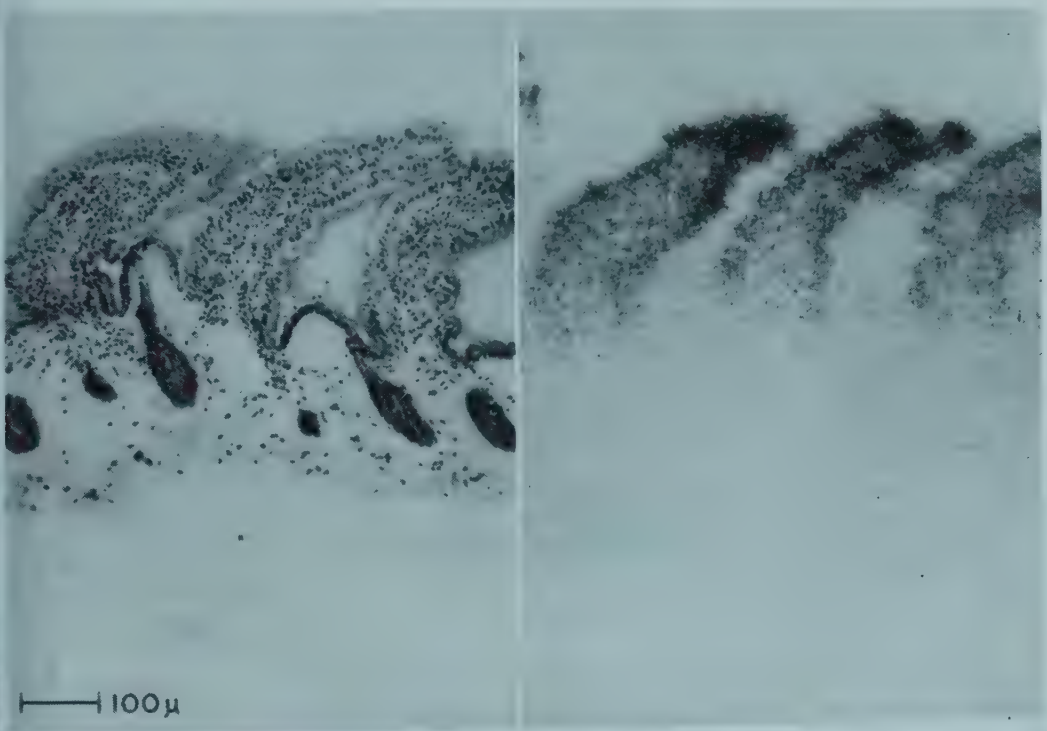

Figure 50. Autoradiograph of a sac of hamster intestine absorbing galactose (5 mM.) for 1 minute. *Left,* Section stained with hematoxylin and eosin. *Right,* Adjacent tissue section exposed to photographic film 4 days. (Kinter: in Proc. 12th Ann. Conf. Nephrotic Syndrome, 1961.)

during tissue accumulation of D-galactose the concentration of this sugar was greater in the epithelial layer than in the rest of the tissue. In this study the tissue was freeze dried and the cell layers separated by microdissection. They inferred from this experiment that the transport system was located on the luminal border of the cell. Similar conclusions were drawn by Kinter[95] with autoradiographic methods developed in his laboratory.[95] This method has considerable resolution, especially when tritiated compounds are employed. Figure 50 shows the accumulation of galactose within the epithelial cells of hamster intestine incubated *in vitro* for one minute in the presence of the C^{14}-labeled sugar. The distinct band of darkening corresponds to the height of the epithelial cell. Similar results were obtained with 3-0-methylglucose, which Csáky has shown to be nonmetabolizable.[49, 53] It is inferred from these data that there is an "entrance pump" at the luminal border of the cell and a permeability barrier at the basal portion of the cell (Figure 51).

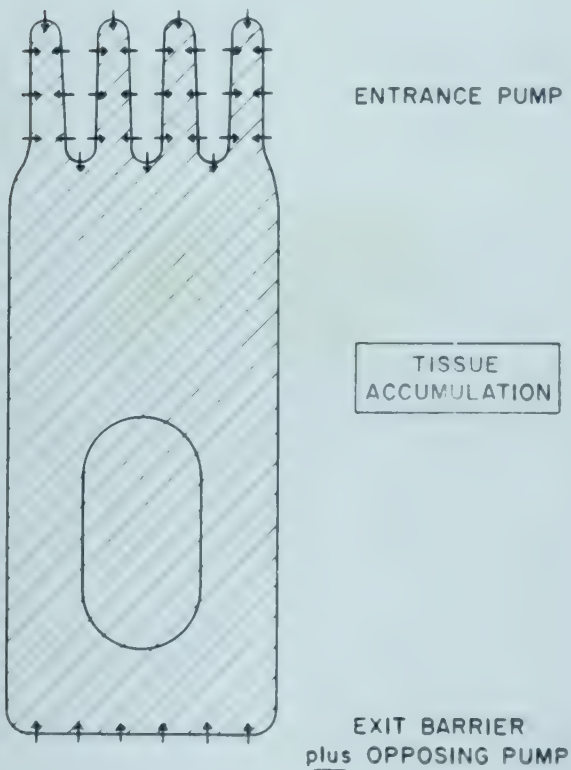

Figure 51. Hypothetical arrangement of sugar transport sites for absorptive cell of the small intestine. (Kinter: in Proc. 12th Ann. Conf. Nephrotic Syndrome, 1961.)

An unexpected finding was the accumulation of sugar in the cells of the crypts of Lieberkühn. The entrance of the sugar into the lumen of the crypt and subsequent transfer into the cell was ruled out by the fact that radioactive inulin did not enter the lumen of the crypt when incubated in a similar manner and by the histological observation that the lumina of the crypts were plugged with mucus. If these observations

are correct, one alternative explanation is that the basal portion of the cell "pumps" sugar from the connective tissue spaces into the cell against a concentration gradient. A working hypothesis is that the active transport system is present along the entire cell membrane, the net movement from lumen to blood being due to the far greater surface area because of the microvilli at the luminal border of the cell (Figure 51). Oxender and Christensen[131] proposed a similar hypothesis in 1959.

FACTORS INFLUENCING GLUCOSE ABSORPTION

Diet

Fasting in rats and other experimental animals leads to a decreased intestinal absorption of glucose as measured *in vivo*. Cori and Cori[34] found that both glucose and fructose absorption were reduced in rats fasted 24 or 48 hours. This general observation was later confirmed by Marrazzi[117] and by Magee.[115] Semistarvation, or reduction in food intake, has the opposite effect. Kershaw, Neame and Wiseman[92] have shown that there is a striking increase in the capacity of the intestine from semistarved rats to absorb both glucose and histidine compared with the control (Table 18). As the absorptive capacity was estimated with the intestine *in vitro* and calculated on a dry weight basis, the authors repeated the experiments, making the absorption measurements *in vivo*. The entire intestine of the semistarved rat had about twice the capacity of the control to absorb glucose. While complete starvation causes a fall in hydrolytic enzyme levels of the intestine, Lojda and Fabry[109] have shown that intermittent starvation results in an increase of both alkaline phosphatase and esterase in the upper intestine. There appears to be a fundamental difference between fasting and semistarvation in their effect on the functional capacity of the small intestine. Whether this is because of an altered pattern of maturation of epithelial cells or some type of adaptive change is a question that requires further study.

Table 18. Effect of Semistarvation and Refeeding on Intestinal Transport

DIETARY REGIMEN		ACTIVE TRANSPORT/100 MG. DRY WEIGHT	
DAYS OF SEMI-STARVATION	DAYS OF SUBSEQUENT FEEDING	GLUCOSE (MG.)	L-HISTIDINE (μMOLES)
(Fed *ad libitum*)		0.80	2.8
9	none	6.6	8.3
9	1	3.9	6.8
9	7	1.2	2.6

Semistarvation in rats was produced by feeding 5 gm. food/animal/day. Subsequent feeding was *ad libitum*. Transport was determined with everted sacs of intestine. Taken from Kershaw, Neame, and Wiseman: J. Physiol., *152*:182, 1960.

Hormonal Changes

There are many profound changes in intestinal absorption associated with altered hormonal balance. Pauls and Drury[135] showed that diabetic animals absorb glucose more rapidly than normal. This was later confirmed.[107, 164, 38] There are conflicting claims on the effect of insulin administration. A recent study of Crane[38] has extended these observations under more controlled conditions than those available previously. Table 19 shows that the transport of the nonmetabolizable sugar, 6-deoxyglucose, was more than twice as great in the alloxan-treated rats as in the controls. No effect of insulin addition *in vitro* was observed.

Table 19. 6-Deoxyglucose Accumulation by Strips of Intestine from Normal and Alloxan-Diabetic Rats

	NUMBER OF ANIMALS	TISSUE CONC. OF 6-DEOXYGLUCOSE (mM.)
Normal	9	2.9
Alloxan-injected	11	6.9

Tissue (0.4-0.6 gm.) was incubated for 21 minutes at 37°C. in 10 ml. bicarbonate-saline containing 0.5 mM. 6-deoxyglucose. Taken from Crane: Biochem. & Biophys. Res. Comm., *4*:436, 1961.

Interest in the relationship between the adrenocortical hormones and absorption arose largely from experiments in the laboratory of Verzár in Switzerland. The first of a series of papers on the reduction of glucose absorption following adrenalectomy was published by Wilbrandt and Lengyel[187] in 1933. These authors also found that xylose absorption was unaffected in adrenaletomized animals, which led the authors to believe that the adrenals control in a rather direct manner the active transport system for sugars. Although there has been some discussion in the literature concerning the role of NaCl supplements to the diets of the adrenalectomized animals, there seems to be considerable support[32, 117] for the original claim of Wilbrandt and Lengyel.

The work of Althausen and his associates has shown a relationship between the thyroid gland and sugar absorption.[3, 4] Removal of the thyroid reduces the absorption rate while the administration of thyroid extract to normal animals increases absorption. Although these experiments were performed *in vivo*, considerable care was taken to control the many variables. This question is of such general interest that it should be explored further with more recent *in vitro* and *in vivo* methods.

There appears to be a definite sex difference in sugar absorption which would tend to implicate the gonadal hormones in the regulation of sugar transport. Deuel et al.[58] found that female rats absorb more

glucose than males. *In vitro* studies of Fisher and Parsons[68, 63] confirm this observation under conditions free of *in vivo* complications of blood flow changes, alterations in gastric emptying, and changes in motility. In addition, Althausen[3] found that ovariectomy significantly reduces the rate of absorption.

A more complete discussion of the extensive literature on this important subject will be found in reviews.[3, 24, 21]

HYPOTHESES FOR THE MECHANISM OF SUGAR ABSORPTION

A variety of hypotheses concerning the mechanism of sugar absorption have been advanced from time to time but only those of current interest will be discussed here. For further details of the older literature or for different points of view the reader may consult other reviews.[167, 173, 169, 36, 185]

Phosphorylation Hypothesis

Wilbrandt has recently reviewed this subject[185] and points out that the concept of chemical alteration of a compound during membrane transport was developed by Höber[83] and Verzár[167] prior to the enunciation of any specific hypothesis. In 1933 Wilbrandt and Laszt[186] proposed that sugars were phosphorylated at the cell membrane as part of a transport mechanism. In the same year Lundsgaard[114] proposed a similar mechanism for sugar absorption by the kidney. The hypothesis was based primarily on the observation that iodoacetate and phlorizin (believed to be specific inhibitors of phosphorylation reactions) inhibited glucose absorption by the kidney and intestine. The hypothesis was extended by Kalckar[90] and Krogh[101] who proposed that when glucose was phosphorylated by hexokinase at the luminal surface of the epithelial cell, the glucose phosphate diffused across the cell and was dephosphorylated by a phosphatase at the serosal border of the cell to become free glucose, which was liberated into the blood. In support of this view it was found that sugar phosphates accumulated in the intestinal epithelium during sugar absorption.[106, 113, 12, 99, 128, 126, 59, 132] Hele[74, 75] and Bissegger and Laszt[14] then presented evidence that the kinase activity in the intestine for different sugars was proportional to their rate of absorption. And finally, alterations in the phosphatase activity in the kidney[118] and intestine[170] in various states seemed to give additional weight to the hypothesis.

Over a period of many years evidence slowly accumulated which seemed to cast doubt on the validity of this theory. The inhibitors, iodoacetate and phlorizin, are now known not to be specific for phos-

phorylation reactions. Iodoacetate reacts with the sulfhydryl groups of many proteins and cannot be considered a specific inhibitor. Lotspeich[111] has recently reviewed the data on phlorizin and concludes that it can act on oxidative reactions[157, 112] at certain concentrations, although its primary site of action is probably permeation mechanisms on cell surfaces. Accumulation of sugar phosphates during absorption cannot be taken as evidence for their participation in transport as many cells increase the concentration of intermediates of metabolism upon the addition of utilizable sugars. The other evidence in favor of the hypothesis either has not been duplicated in other laboratories as in the case of the kinases[162] or is amenable to alternative explanations as with the phosphatase changes. Thus, the evidence on which the theory was based has proved to be inadequate.

Perhaps the most compelling evidence against the hypothesis of phosphorylation is the accumulated data on the specificity of the sugar transport system of the hamster (see previous section). The data indicates that the specificity requirement for transport includes six or more carbon atoms, a D-pyranose ring structure, and an intact hydroxyl group at carbon 2. Phosphorylation of a hydroxyl group at carbons 1, 3, 4, 5, or 6 has been eliminated and such a combination at carbon 2, although not excluded, would be a very unusual reaction.

Further evidence that glucose-6-phosphate was not an intermediate in glucose absorption was provided by Landau and Wilson.[102] In these studies radioactive galactose was used to label the glucose-6-phosphate pool within the tissue and the proportion of transported glucose passing through this pool was estimated by the radioactivity of the transported glucose recovered on the serosal side of intestinal segments. This calculation indicated that less than 10 per cent of the transported glucose could have passed through this pool. Although this experiment was of an indirect type, it suggested that phosphorylation at carbon 6 was not involved in glucose absorption. One variation of the general phosphorylation hypothesis involves the conversion of glucose to two triose phosphates followed by recondensation to hexose. This possibility was considered by Wilson and Vincent[195] as lactic acid was found during glucose and fructose absorption both *in vitro*[185, 197, 198, 190, 130] and *in vivo*.[5, 97, 98] Conclusive evidence against this triose hypothesis, however, has been presented for the intestine[195, 165, 72] and the kidney.[22]

Mutarotation

In 1954 Keston[93] suggested an interesting mechanism for sugar transport by the kidney and small intestine. He found that the mutarotase present in the kidney had a substrate specificity similar to that of the kidney's sugar absorption (glucose, galactose, and xylose) and that the

enzyme was inhibited by phlorizin. The fact that 1-deoxyglucose as well as α and β-methylglucoside (compounds which cannot mutarotate) are transported by the intestine makes this theory unlikely.

Other Chemical Mechanisms

Crane and Krane[40] have excluded some other types of specific chemical reactions during transport by a series of ingenious experiments. The substitution of CH_2OH for the H at carbon 2 (2-c-hydroxymethyl glucose) does not alter the transport of the molecule, indicating that oxidation-reduction at C_2 could not occur. The possibility that the hydroxyl group at carbon 2 was involved in some chemical reaction during transport was also considered by Crane and Krane.[40] They performed experiments with 1, 5-anhydro-D-glucitol in the presence of H_2O^{18} and with glucose-2-O^{18} in the presence of H_2O. During intestinal transport of these sugars no exchange of O^{18} took place. These experiments eliminate dehydration-rehydration or certain other reactions which would entail the loss of oxygen from carbon 2.

Direct Coupling of Na Pump and Sugar Transport

The observations of Riklis and Quastel[147] and others[23, 43, 47, 52, 54, 151] that Na is necessary for glucose transport have stimulated speculation on the role of this ion in sugar absorption. Crane, Miller, and Bihler[43] have recently shown a direct correlation between Na concentration in the medium and sugar transport. A direct coupling of the sodium transport system to that for sugars was suggested to account for this observation. The relationship between Na^+ and sugar transport is an extremely interesting one and bears further exploration.

Pinocytosis

Pinocytosis has been considered as one possible mechanism for transport of sugars. Recently this type of mechanism has been shown to be involved in sugar permeability of adipose tissue in response to insulin.[10] In the intestine, however, sugar absorption is not stimulated by insulin or other proteins. Although pinocytosis cannot be excluded from consideration there is no evidence at present to support such a hypothesis.

Current Views

Attempts to discover specific chemical alterations in the glucose molecule during intestinal transport have thus far met with failure. This has suggested that perhaps transport does not involve formation and splitting of a covalent bond but simply an adsorption to and desorption from some type of membrane carrier probably located in the plasma

membrane on the luminal border of the cell. With the modern tools of the biochemist available, it now appears feasible to attack the problem from many different aspects. Such studies are under intensive investigation in many laboratories and perhaps in the not too distant future some concrete information will be uncovered concerning the biochemical mechanism of sugar transport by the intestine.

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CHAPTER 5

Amino Acids

SOURCE OF PROTEIN PRESENTED TO THE GI TRACT

Protein Intake

Protein is one of the major foodstuffs in the animal diet and is essential for life. A variety of amino acids cannot be synthesized by the animal body and must therefore be provided in the diet in the form of protein. A 70 kg. man must eat approximately 45 gm. of protein per day to maintain nitrogen balance. This quantity of protein is readily digested and completely absorbed in a normal individual.

Endogenous Protein

The protein molecules presented to the small intestine consist not only of those derived from the diet but those of endogenous origin such as the enzymes of the digestive secretions and desquamated epithelial cells. Nasset et al.[52] have studied the amino acid composition of the intestinal contents of dogs fed with and without proteins. A similar distribution of free amino acids was observed in the lumen when the animal was fed a protein with an unusual amino acid pattern or when the dog had been fasting. The authors concluded that considerable endogenous protein diluted the dietary protein. Additional experiments were performed by Nasset and Ju[51] who estimated that when dogs or rats were fed a meal of labeled casein there was a three- to eightfold dilution of

the ingested casein in the lumen of the intestine. The source of the endogenous protein is mainly the protein of the gastrointestinal secretions. If 7 liters of secretions, an estimate for man, contain an average of 2 per cent protein, then 140 gm. of protein would be added to the intestinal contents in this form. Desquamation of the epithelial lining of the G.I. tract in 24 hours might be about 250 gm., according to the estimate of Leblond and Walker,[40] which might add an additional 25 gm. of protein. If the total intake of protein per day is assumed to be about 50 gm., this will be diluted in the intestinal tract by a factor of about 3. The dilution undoubtedly varies with different conditions in different animals but is certainly a significant feature of protein digestion and absorption in most animals.

PROTEIN ABSORPTION IN THE FETUS AND NEWBORN

The fetal and newborn animal of most mammalian species absorbs intact protein molecules from its small intestine by a process of pinocytosis.[12] This capacity to absorb large molecules and particulate material ceases shortly before or after birth (depending on the species) and the adult impermeability of the intestine appears. In the human this change occurs before birth while in the rat the juvenile state persists until weaning, at about three weeks of age. The epithelial cell of the adult mammal is impermeable to intact protein molecules. The absorption of small amounts of antigens and other proteins is well known but it is quantitatively insignificant. Chapter 10 deals with this interesting problem in considerable detail.

FORM IN WHICH PROTEINS ARE ABSORBED

(Peptides versus Free Amino Acids)

Because of the difficulties in estimating amino acids before 1911 the earlier workers could not decide whether proteins were absorbed intact, as peptides, or as free acids.[21] With the development of the nitrous acid method for alpha amino nitrogen determination, Van Slyke and Meyer[70] were able to demonstrate a rise in free amino acid nitrogen concentration in the blood after feeding beef to dogs (Table 20). A small but significant rise in blood amino acid level was observed during circulation through the absorbing small intestine.

London,[45] while confirming the observation of an increase in amino acids in portal blood during absorption in dogs, claimed to identify considerable quantities of peptides in portal blood after a meal. Methods

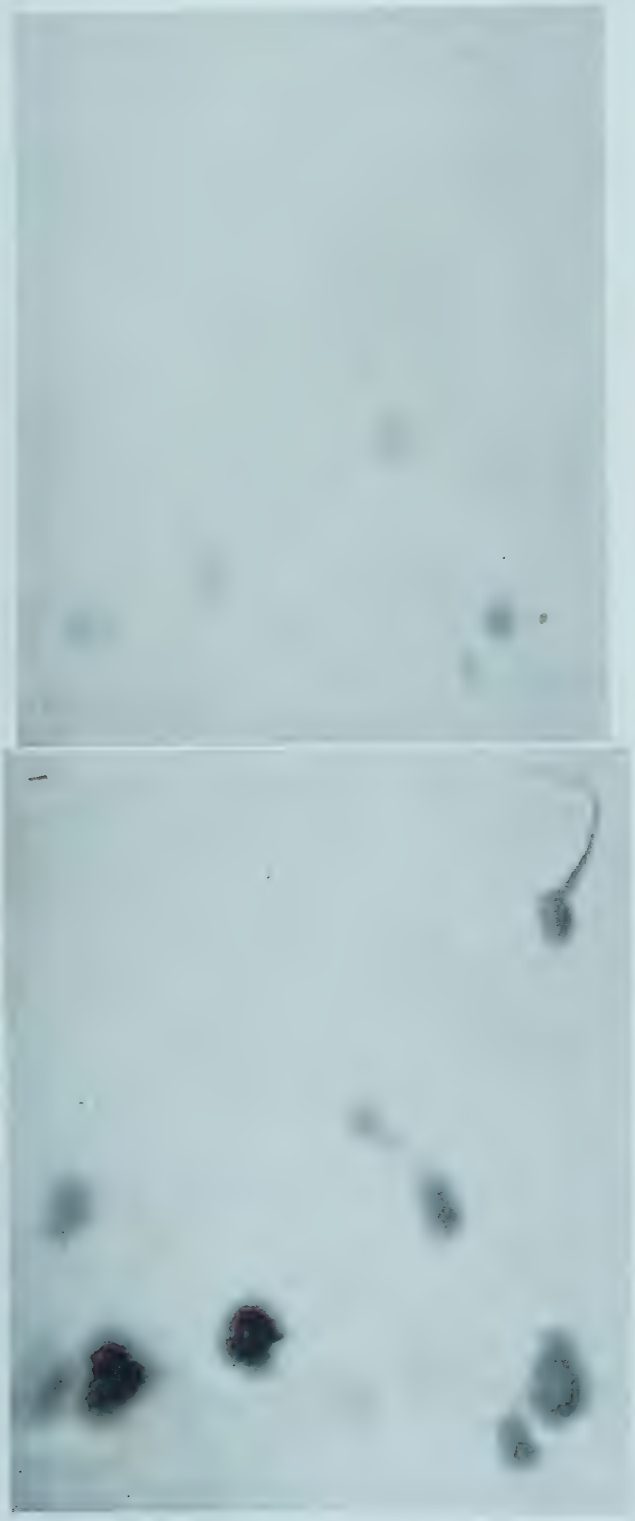

Figure 52. Appearance of free amino acids in portal vein blood following a protein meal. Two-dimensional paper chromatograms sprayed with ninhydrin to visualize amino acids. Samples of portal blood were taken from a fasting dog (upper) and from the same dog 2.5 hours following ingestion of 400 ml. of 25 per cent human serum albumin (lower). (Dent and Schilling, Biochem., J., *44*, 1949.)

for the estimation of peptides at that time were entirely unsatisfactory and not until the advent of chromatography was it possible to reinvestigate the problem with reliable analytical procedures. Dent and Schilling[16] and Christensen[10] in 1949 made a careful study of the amino

Table 20. Effect of a Protein Meal on Amino Acid Content of Blood

TIME	VESSEL	AMINO ACID NITROGEN (MG./100 ML.)	
		DOG A	DOG B
Before feeding	Femoral artery	3.7	5.2
5 hr. after feeding	Femoral artery	8.6	9.9
5 hr. after feeding	Mesenteric vein	9.5	10.2

Dogs fasted 24 hours and then fed 1 kg. of fresh beef. Taken from Van Slyke and Meyer: J. Biol. Chem., *12*:399, 1912.

acid pattern found in the portal vein of dogs after ingestion of various types of protein. Figure 52 shows two paper chromatograms of portal blood plasma from a fasted dog and the dog five hours after being fed human serum albumin. There was a striking increase in many of the free amino acids. Similar results were obtained with casein and ground beef. No peptides associated with protein absorption were found in the portal blood. A small amount of amino acid conjugate was present in blood but was unrelated to absorption. These observations have been confirmed by a variety of investigators.[59, 18, 17, 41, 25, 82] The elegant column chromatography of Moore and Stein has recently been applied to this problem by Levenson et al.[41] with entirely similar results.

In vitro experiments have confirmed the view that peptides are poorly absorbed. Agar, Hird, and Sidhu[1] found that when leucylglycine, glycylglycine, or glycylglycylglycine was placed on the mucosal side of an *in vitro* preparation of rat intestine very little peptide appeared on the serosal side while large amounts of free glycine were present on both sides. Another series of peptides was studied by Wiggans and Johnston,[73, 74, 36] who confirmed the observation that little peptide crossed the gut wall *in vitro*. Newey and Smyth[57] found very little absorption of peptides with both *in vivo* and *in vitro* methods. Of all the compounds tested, the glycine peptides were most resistant to hydrolysis and only with these compounds was any detectable peptide found on the serosal side. An incidental finding[74, 36] was the presence of considerable peptidase activity on the serosal side of *in vitro* preparations and in the peritoneal cavity of intact animals. Most peptides clearly have a short half-life either in the lumen of the intestine or in the body.

NEUTRAL AMINO ACID TRANSPORT SYSTEM

The Demonstration of Active Transport

The fact that amino acids were readily absorbed by the intestine was known for many years but until 1950 the evidence for selective absorption was inconclusive. On the one hand Höber and Höber[27] showed that

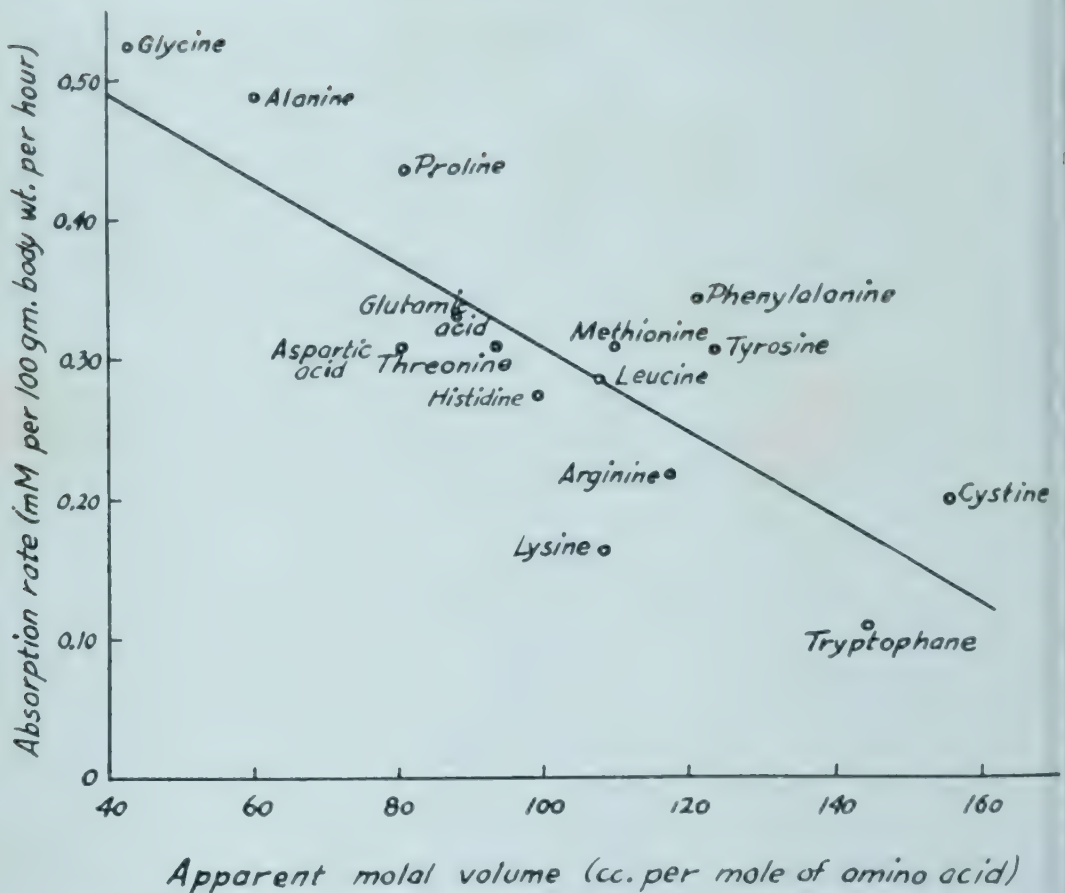

Figure 53. The relation between the rate of absorption of amino acids from the intestine of the chick and the apparent molal volume of the amino acid. (Kratzer: J. Biol. Chem., *153*, 1944.)

amino acids were absorbed faster than polyhydric alcohols of similar molecular volume and unlike the alcohols the amino acids showed saturation phenomena during absorption at high concentrations. These authors concluded that some type of selective absorption process was involved. On the other hand, the rate of absorption of various amino acids in the chick varied inversely with the molecular volume of the compound (Figure 53). This data led to the conclusion that all amino acids were absorbed by simple diffusion. In addition, no consistent difference in absorption rate was found[9, 7] between the two stereoisomers.

The crucial experiments for the demonstration of a special process for the intestinal absorption of L-amino acids were performed by Wiseman and his collaborators[19, 13, 23, 78, 79] in a series of *in vivo* and *in vitro* experiments. An important innovation was the use of specific enzymatic methods to determine the two enantiomorphs of the amino acids. The first series of experiments[19, 23] involved the study of the absorption of a racemic mixture of an amino acid from a washed loop of intestine in

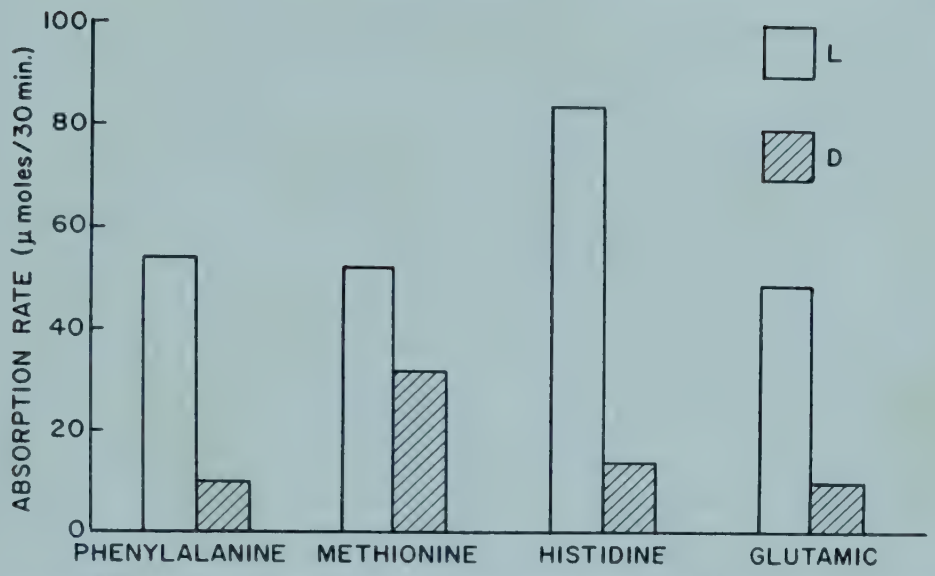

Figure 54. Selective absorption of stereo-isomers of amino acids from loops of rat intestine *in vivo*. One ml. of a racemic mixture of an amino acid (0.2-0.4M.) was introduced into a loop of intestine of an anesthetized rat. After 0.5 to 1 hour, loss of amino acid from the loop determined by enzymatic methods. (Drawn from the data of Gibson and Wiseman: Biochem. J., *48*:426, 1951.)

an anesthetized rat. After 30 to 60 minutes the amino acid remaining in the loop was washed out and the L- and D-isomers estimated separately with the L- amino acid oxidase from Neurospora, L-amino acid decarboxylases and D-amino acid oxidase from kidney. The L-isomer was absorbed more rapidly than the D-enantiomorph in all of the 13 amino acids tested. The results of four of the experiments by Gibson and Wiseman[23] are given in Figure 54. The most striking case was that of histidine in which the L-enantiomorph was absorbed six times as fast as the D-isomer. These results were confirmed in the guinea pig,[22] dog,[13] and man.[39]

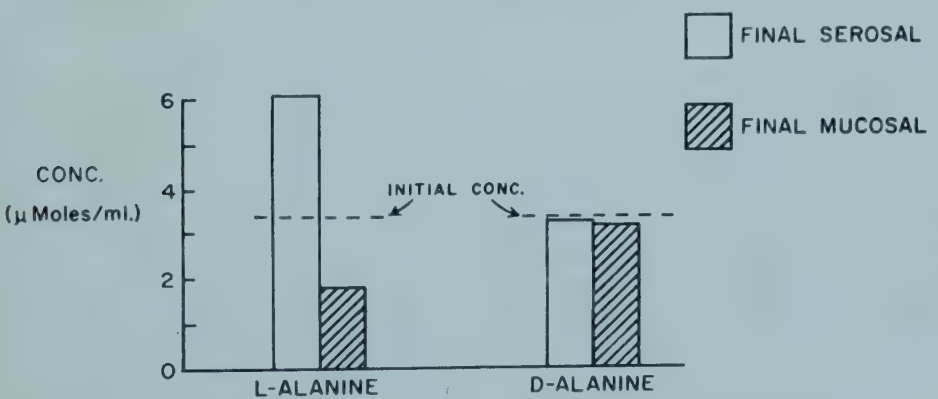

Figure 55. Active transport of L-alanine across rat intestine *in vitro*. Racemic mixture of alanine was placed at equal concentration on both sides of rat intestine. Following an hour's incubation the amino acids on the two sides were determined by the specific L-amino acid oxidase and D-amino acid oxidase. (Drawn from the data of Wiseman: J. Physiol., *120*:63, 1953.)

The first unequivocal demonstration of the active transport of L-amino acids was an *in vitro* experiment by Wiseman in 1951.[78] When a racemic mixture was added to both sides of the intestinal wall the L-stereo-isomer moved across the wall against a concentration gradient while the D-isomer did not (Figure 55). This important series of experiments stimulated considerable interest in this area of investigation.

Agar, Hird, and Sidhu[1] confirmed and extended the stereospecificity of amino acid transport with *in vitro* methods. In addition, they added an important improvement in methodology for the study of intestinal absorption.[2] They found that, when tissue segments were incubated in solutions containing amino acids, the tissue concentration of amino acid rose to a value considerably higher than that in the medium. Tissue accumulation occurred with L- but not D-histidine (Table 21) and was inhibited by cyanide and dinitrophenol. As uptake into the tissue could be blocked by a variety of agents but release from the tissue was not affected it was concluded[2] that active transport occurs from the lumen into the epithelial cell while release from the opposite border of the cell occurs by diffusion.

Table 21. ACCUMULATION OF L- AND D-HISTIDINE BY SEGMENTS OF RAT INTESTINE IN VITRO

ISOMER USED	INITIAL EXTERNAL CONC. mM	FINAL EXTERNAL CONC. mM	FINAL CONC. IN TISSUE WATER mM	RATIO OF CONC. IN TISSUE TO MEDIUM
L-	5	3.82	11.3	3.0
L-	10	8.0	16.5	2.1
L-	20	17.7	23.6	1.3
D-	20	18.4	6.3	0.3

About 2 gm. of rat intestine, cut into 0.5 cm. lengths, were incubated in 20 ml. of Krebs bicarbonate saline containing histidine for 1 hr. at 38°C. Data taken from Agar, Hird, and Sidhu: Biochim. et biophys. acta, *14*:80, 1954.

Competition Between Neutral Amino Acids for a Common Carrier

Wiseman[80] showed that a number of neutral amino acids competed with each other for transport with some being better inhibitors than others. He also found that the charged amino acids, lysine, ornithine, and glutamic acid, had no inhibitory effect on the transport of histidine and methionine. These observations have been confirmed by numerous investigators.[60, 3, 58, 34, 24, 53, 20, 66, 43, 44] Elegant quantitative data on competitive inhibition of monoiodotyrosine by phenylalanine were presented by Nathans, Tapley and Ross.[53] Figure 56 shows that transport data can be treated in a manner similar to that for enzyme kinetics. Recently Finch and Hird[20] studied the effect of concentration on amino acid transport and calculated the concentration at which half the maximal

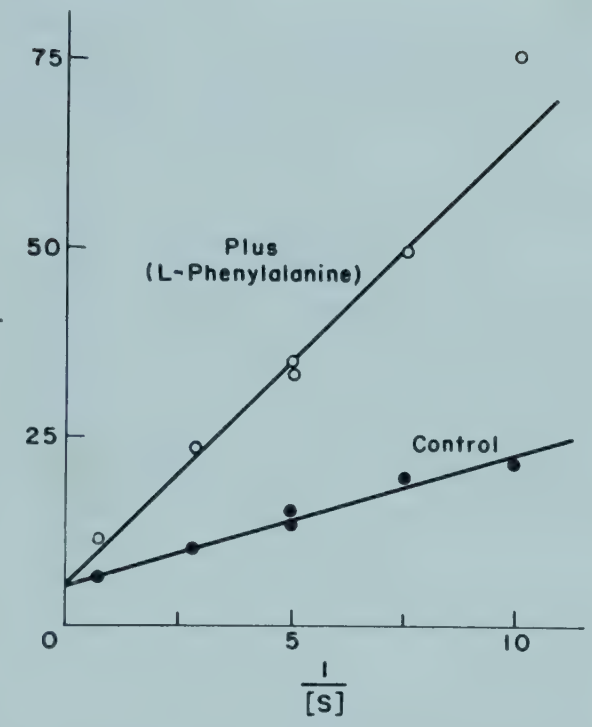

Figure 56. The competitive inhibition of L-monoiodotyrosine transport by L-phenylalanine. Sacs of rat intestine were incubated in radioactive L-monoiodotyrosine with or without L-phenylalanine. Data is given by the graphical method of Lineweaver and Burke. (Nathans et al.: Biochim. et biophys. acta, *41*, 1960.)

transport rate was obtained (K_m of enzyme kinetics). A remarkably good correlation exists between the apparent affinity of amino acids for the transport system as estimated by Finch and Hird and their effectiveness as inhibitors of transport (Table 22). It is inferred from studies of this type that all of the neutral amino acids share a common step in their transport, probably the membrane carrier.

Table 22. COMPETITION BETWEEN AMINO ACIDS FOR ACTIVE TRANSPORT

INHIBITOR	K_t* OF INHIBITOR	INHIBITION OF TRANSPORT OF	
		L-MONOIODOTYROSINE† ($1x10^{-7}$ M.)	L-HISTIDINE‡ ($1x10^{-2}$ M.)
($x10^{-2}$ M.)	($x10^{-3}$ M.)	PER CENT	PER CENT
Glycine	27	0	22
L-Alanine	5	50	34
L-Valine	2.1	89	62
L-Isoleucine	1.2	97	70
L-Methionine	0.9	100	86
L-Leucine	0.7	97	91

* K_t is analogous to the K_m of enzyme kinetics. Data from Finch and Hird: Biochim. et biophys. acta, *43*:278, 1960.

† From Nathans, Tapley, and Ross: Biochim. et biophys. acta, *41*:271, 1960.

‡ From Agar, Hird, and Sidhu: Biochim. et biophys. acta, *22*:21, 1956.

Effect of Metabolic Inhibitors

Amino acid absorption in adult intestine requires aerobic pathways involving oxidative phosphorylation, as dinitrophenol,[1, 22, 58] cyanide,[2]

and anaerobic conditions[22] inhibit transport. Winter[77] has shown that fluoroacetate inhibits glycine absorption in rats at concentrations which do not affect glucose absorption. Low temperature reduces the ratio of L- to D-amino acids absorbed by loops of gut *in vivo*.[47]

Specificity of the Transport System

Optical Specificity: One of the striking features of the amino acid transport system in the mammalian small intestine is its preference for the L-stereo-isomer. In the early studies of Wiseman both isomers were present together and inhibition of D-amino acid transport by the presence of the L-isomer could not be ruled out. Since that time the two isomers of amino acids have been tested separately in a number of cases (Table 23). In five out of the six amino acids tested no transport against a

Table 23. Active Transport of D- and L-Amino Acids

Amino Acid	Conc. (mM.)	Method	Transport μmoles/100mg. tissue/hr.	
			L-isomer	D-isomer
Histidine*	20	Tissue accumulation	+	0
Phenylalanine†	15	Tissue accumulation	+	0
Tyrosine‡	3	Sac	4.9	0
Alanine‡	5	Sac	4.6	0
Tryptophan‡	5	Sac	1.8	0
Methionine‡	5	Sac	4.7	1.9

* Agar, Hird, and Sidhu: Biochim. et biophys. acta, *14*:80, 1954.
† Agar, Hird, and Sidhu: Biochim. et biophys. acta, *22*:21, 1956.
‡ Lin, Hagihira, and Wilson: Am. J. Physiol., in press.

concentration gradient of the D-enantiomorph could be detected. D-methionine, however, was shown to be transported by Jervis and Smyth,[35] indicating that the stereospecificity is not absolute. The available data suggests that absorption of D-amino acids involves the same carrier system as the L-enantiomorphs, although the latter possess a much greater affinity for the system. This is supported by the fact that L-amino acids powerfully inhibit the penetration of the D-isomers[3, 20, 33a] and at high concentrations some D-amino acids inhibit the transport of certain L-amino acids.[20, 33a, 58]

As the transport system for neutral amino acids can distinguish between the two optical isomers, at least three groups on the asymmetric α-carbon of the amino acid must interact with the carrier.

Carboxyl Group: When the charge on the carboxyl group was removed by the formation of an ester (e.g., methyl ester of L-histidine), its capacity to be transported was lost.[43] Likewise, the reduction of the carboxyl group to an alcohol, as in L-histidinol, led to an inactive compound. The importance of the carboxyl group was further supported by

the observation that L-tyrosine, but not its decarboxylated product, L-tyramine, inhibited amino acid transport.[53] In addition, the substitution of a sulfonic acid group for the carboxyl group reduced its affinity for the carrier.[53]

α-AMINO GROUP: The removal of the charge on the amino group of L-histidine by acetylation produced a compound not actively transported by hamster intestine.[43] This agreed with the observation[53] that the acetyl derivatives of glycine, valine, and methionine did not possess any affinity for the transport system. The replacement of the amino group with a hydroxyl group resulted in an inactive compound. Thus, L-alanine was actively transported[79] while DL-lactate was not.[75] The observation that β-alanine was neither transported[43] nor an inhibitor[53] of transport lends further support to the hypothesis that the amino group participates in interaction with the carrier in a stereospecific manner. The N-methyl derivatives of glycine (sarcosine, N, N-dimethylglycine, and betaine) do not inhibit transport of glycine (Table 24). A number of amino acid derivatives which lack the amino group (urocanic acid, p-hydroxyphenylpyruvate, and phenylpyruvate) were also tested and all found to be inactive.[43]

Table 24. EFFECTS OF AMINO ACIDS ON GLYCINE TRANSPORT

INHIBITOR 30mM.	PER CENT INHIBITION OF GLYCINE TRANSPORT
L-alanine	79
Sarcosine	11
N-Dimethylglycine	<10
Betaine	<10

Tissue accumulation of C^{14}-glycine was estimated in the presence and absence of "inhibitor" amino acids. Initial concentration of glycine in the medium was 3 mM. An inhibition of less than 10 per cent was not considered significant.

Further evidence implicating the amino group is the requirement for pyridoxal phosphate or its derivatives. Animals made pyridoxine deficient by deoxypyridoxine,[30-32] penicillamine,[4, 69] or B_6-deficient diet[32, 43] showed defective intestinal absorption of amino acids. Figure 57 shows an experiment by Jacobs and Hillman[32] in which deoxypyridoxine treatment reduced the capacity of rat intestine to absorb L-methionine. Akedo, Sugawa, Yoshikawa, and Suda[4] performed the following elegant experiment. L-Histidine absorption was measured *in vivo* in pyridoxine-deficient rats. After a 20-minute control period vitamin B_6 was injected intravenously and within 10 minutes the rate of L-histidine absorption had increased to the normal absorption rate (Figure 58). These experiments strongly suggest that pyridoxal phosphate is involved in the intestinal absorption of neutral amino acids. This fact, together with the

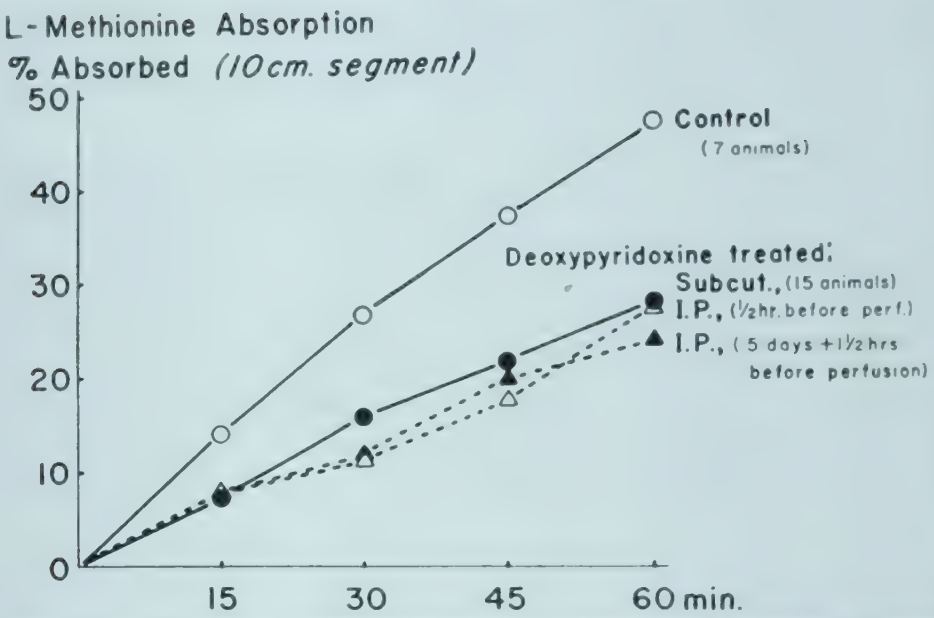

Figure 57. The effect of deoxypyridoxine on L-methionine absorption. Deoxypyridoxine HCl (0.2 mg.) was given daily for 4 to 10 days (closed circles) or as indicated. (Jacobs and Hillman: J. Biol. Chem., *232*, 1958.)

observation that di- and tri-subsituations on the α-nitrogen possess no affinity, suggest that a Schiff base may be involved in transport of neutral amino acids.

α-HYDROGEN: The α-hydrogen cannot be replaced by a methyl group without a reduction in the rate of transport.[76, 43] Two of these compounds, α-aminoisobutyric acid and α-methylmethionine, are slowly trans-

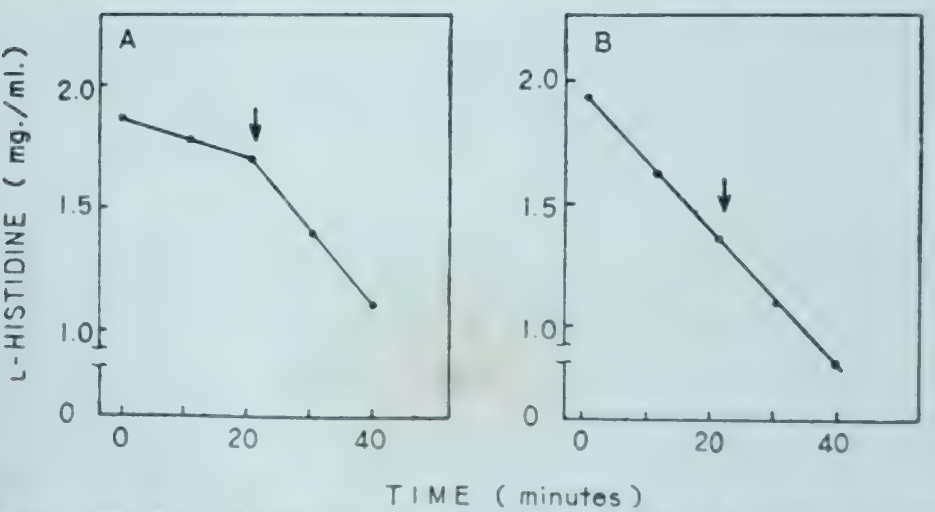

Figure 58. Effect of intravenous pyridoxine on L-histidine absorption in vitamin B_6-deficient rats. Animal A was made B_6 deficient by administration of L-penicillamine while control animal B was given vitamin B_6. Absorption of L-histidine was carried out *in vivo* for 20 minutes and then 500 μg. of B_6 was injected into the caudal vein at the point indicated by the arrow. The ordinate is the concentration of amino acid in the intestinal loop (fall in concentration indicates absorption). (Akedo et al., J. Biochem., *47*, 1960.)

ported and have a weak inhibitory effect on the transport of other amino acids.[43] Christensen[11] has studied cycloleucine (1-amino, 1-carboxyl-cyclopentane), which is transported but has less affinity for the transport system than L-leucine.

SIDE CHAIN: A wide variety of natural and unnatural L-amino acids with neutral side chains are transported by the small intestine.[79, 81 29, 65, 44, 43] Figures 59 and 60 show the structures of the side chains of amino acids transported by the intestine of the golden hamster. It is clear that the carrier system is exceedingly nonselective in side chains.

In contrast to the latitude permitted by the carrier system in structural variations of the side chain, the introduction of a charge into the side chain abolishes the affinity of the compound for the transport system, whether the charge be positive or negative. Wiseman showed[80] that L-lysine and L-ornithine did not inhibit the transport of a number of neutral amino acids. L-Citrulline, which is closely related to ornithine but without a charged side chain, is actively transported by the intestine and inhibits the carrier for neutral amino acids. L-Glutamic and L-aspartic acids do not inhibit, while L-glutamine and L-asparagine are good inhibitors. The last two amides might be expected to be transported but they are deaminated by the intestine and are therefore difficult to test for transport. The γ-methyl ester of L- glutamic acid (a neutral derivative of glutamic acid) was well transported by hamster intestine and a good inhibitor of glycine transport.[43] L-Tyrosine could be converted into a

1. $H-$
2. CH_3-
3. $\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>\!CH-$
4. $CH_3-CH_2-\underset{CH_3}{\overset{}{>}}CH-$
5. $CH_3-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-$
6. $CH_3-CH_2-CH_2-CH_2-$
7. $CH_3-S-CH_2-CH_2-$
8. $CH_3-CH_2-S-CH_2-CH_2-$
9. $HO-CH_2-$
10. $HO-\underset{\displaystyle CH_3}{\underset{|}{CH}}-$
11. $CH_3-O-\overset{\displaystyle O}{\overset{\|}{C}}-CH_2-CH_2-$
12. $NH_2-\overset{\displaystyle O}{\overset{\|}{C}}-NH-CH_2-CH_2-CH_2-$

Figure 59. Amino acids actively transported by the intestine (aliphatic side chains). 1, glycine; 2, alanine; 3, valine; 4, leucine; 5, isoleucine; 6, norleucine; 7, methionine; 8, ethionine; 9, serine; 10, threonine; 11, glutamate methyl ester; 12, citrulline. All of the compounds first tested by Wiseman[79, 81] except 6, 8, 11, and 12 which were tested by Lin et al.[43]

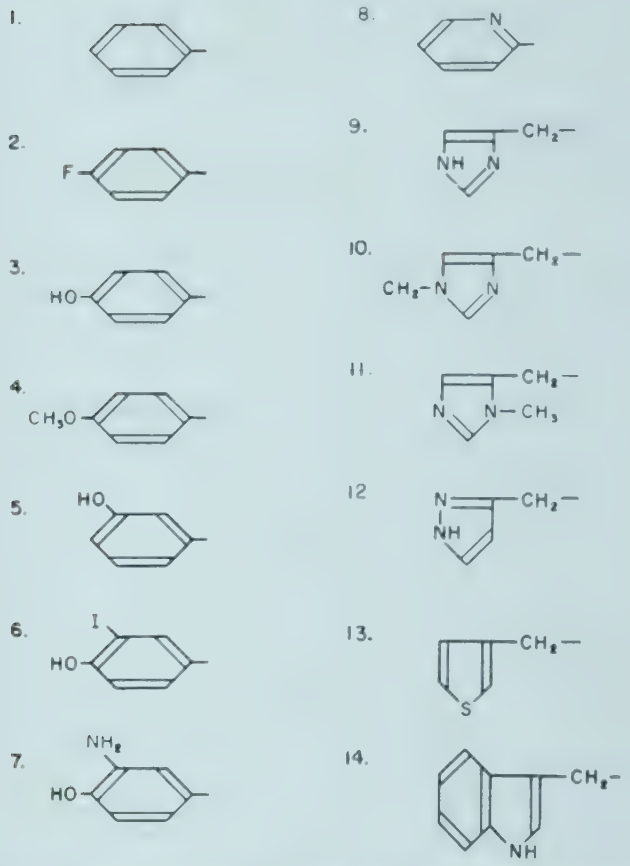

Figure 60. Amino acids actively transported by the intestine (aromatic and heterocyclic side chains). 1, phenylalanine; 2, p-fluorophenylalanine; 3, tyrosine; 4, O-methyltyrosine; 5, m-tyrosine; 6, 3-iodotyrosine; 7, 3-aminotyrosine; 8, pyridylalanine; 9, histidine; 10, 1-methylhistidine; 11, 3-methylhistidine; 12, 3-pyrazolealanine; 13, thionylalanine; 14, tryptophan. Compounds 1 and 9 tested by Wiseman,[79, 81] 4 by Huang,[29] 6 by Nathans, Taplev, and Ross,[53] 3 and 5 by Lin and Wilson,[44] 14 by Spencer and Samiy,[65] the remaining compounds by Lin et al.[42, 43]

nontransported compound by inducing ionization of the phenolic group through the introduction of two bromine atoms into the ring (L-3, 5-dibromotyrosine). The observation that L-djenkolic acid, which has both a positive and a negative charge in its side chain, was also not transported[43] further indicates that the important condition for transport of an amino acid by this carrier is that it must be charge-free.

Huang has studied an interesting series of tyrosine derivatives in both the kidney and the gut.[28, 29] He found that those with neutral side chains were actively absorbed by the gut and resorbed by the renal tubule; those with ionized phenolic groups were not transported by the gut and were secreted by the renal tubule (presumably by the weak acid secretory system).

Lin, Hagihira, and Wilson[43] have emphasized the relationship between the affinity of the carrier system for an L-amino acid and the nonpolar character of the side chain. Table 25 shows that increasing the

Table 25. Correlation Between "K_t" and Polarity of the Side Chain

AMINO ACID	"K_t"*	SOLUBILITY ETHANOL TO WATER†
	(x10-3M.)	(x10-4M.)
Glycine	27	1.3
L-Alanine	5	4.5
L-Valine	2.1	23
L-Leucine	0.65	75

* Finch and Hird, Biochim. et biophys. acta, *43*:278, 1960.
† Cohn, McMeekin, Edsall, and Weare, J. Am. Chem. Soc., *56*:2270, 1934.
K_t is analogous to the K_m of enzyme kinetics.

length of the alkyl side chain for a series of amino acids increases the ethanol-water solubility ratio and increases the affinity (measured by K_t). In this connection it is pertinent to point out that a charge in the side chain not only prevents active transport of most of the amino acids but also abolishes their affinity for the carrier. These observations suggest that solubility of the side chain in the lipid-rich membrane is a decisive factor in permitting the carboxyl group, amino group, and α-hydrogen to gain access to the active site of the carrier. A pictorial representation of this view is given in Figure 61.

Mechanism of Amino Acid Transport

The involvement of pyridoxal phosphate is strongly suggested by the data given previously.The possibility of decarboxylation and recarboxylation as a mechanism was eliminated by Lin et al.,[43] who showed that glycine-1-C^{14} does not lose its carboxyl carbon during transport. Tapley et al.[68, 26] have suggested that glucuronide formation may be involved in active transport. Further evidence is needed to support this suggestion.

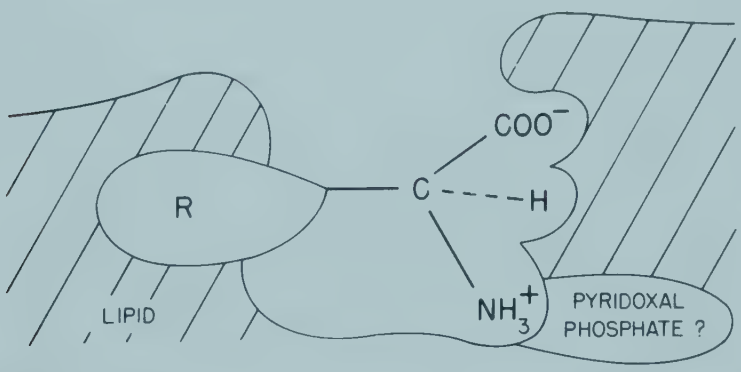

Figure 61. Hypothetical interaction of neutral amino acid with the membrane carrier.

"BASIC" AMINO ACID TRANSPORT SYSTEM

Active transport of basic amino acids by the small intestine was not seriously considered until very recently. It has been previously shown that L-lysine was absorbed faster than the D-isomer *in vivo*[23] and that pyridoxine stimulated L-lysine absorption in B_6-deficient rats.[4] Furthermore, absorption *in vitro* seemed to be more rapid than would be anticipated by diffusion.[18a] The inference from these experiments, however, was not clear because the workers were unable to demonstrate active transport of L-lysine and L-ornithine when tested under the same conditions that clearly indicated transport of neutral amino acids.[80]

An important piece of information was provided by Milne, Asatoor, and Loughridge[49] who showed that in patients with cystinuria the intestinal absorption of lysine and ornithine was defective. They reported that when these two basic amino acids were fed to cystinurics considerable amounts could be recovered in the feces, while similar amounts were completely absorbed by normal individuals. It was inferred that in cystinuria the genetic defect in the transport of cystine, lysine, ornithine, and arginine by the kidney tubule was shared by the small intestine.

These observations stimulated Hagihira et al.[23a] to reinvestigate the question of transport of these four amino acids with *in vitro* methods. When everted sacs of hamster intestine were incubated with low concentrations (1 mM.) of amino acid both inside and outside, net transport against a concentration gradient was observed for all three basic amino acids (Table 26). It was already known from the work of Neil[56] that L-cystine was actively transported. It might be noted that the transport of basic amino acids is against an electrical as well as a chemical gradient, as the serosal side of the gut is positive with respect to the mucosal side.[62] The maximal rates of transport of these amino acids was 1/10 to 1/20 the transport rate for some of the neutral amino acids, e.g., glycine and L-alanine.[81] The very low capacity of these basic amino acids for transport explains the difficulty in demonstrating net transport with high initial concentrations.[78]

It was then shown that cystine and the three basic amino acids share the same transport system. Ornithine, arginine, and cystine inhibit

Table 26. ACTIVE TRANSPORT OF "BASIC" AMINO ACIDS

AMINO ACID	FINAL CONC. RATIO SEROSAL: MUCOSAL	NET TRANSPORT μMOLES/100 MG. TISSUE/HR.
L-Lysine (1mM.)	5.1	0.77
L-Arginine (1 mM.)	1.5	0.21
DL-Ornithine (2 mM.)	2.0	0.51
L-Cystine (0.2 mM.)	2.5	0.25

Everted sacs of hamster intestine incubated one hour at 37°C. Taken from Hagihira Lin, Samiy, and Wilson: Biochem. & Biophys. Res. Comm., *4*:478, 1961.

L-lysine transport and ornithine, arginine, and lysine inhibit cystine transport.[23a, 23b] Previous studies had shown that the three basic amino acids have no inhibitory effect on the transport of L-histidine[3, 80] or iodotyrosine.[53] In addition, L-lysine did not inhibit the transport of L-isoleucine[20] or L-methionine.[78] Although the basic amino acids do not inhibit the neutral amino acid transport system, some neutral amino acids can inhibit the "basic" transport system. Thus, L-methionine has some inhibitory effect on L-lysine transport, although less than its effect on glycine transport.

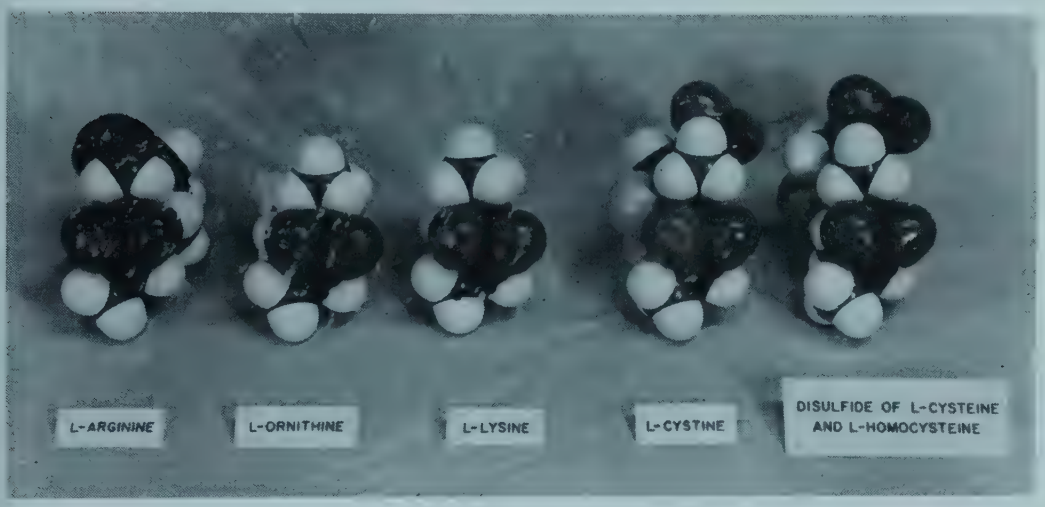

Figure 62. Structures of arginine, ornithine, lysine, cystine and the disulfide of cysteine and homocysteine. All of these compounds appear in the urine of patients with cystinuria.

The combination of the data on patients with cystinuria and that obtained with the isolated intestine convincingly suggests that there is a separate transport system in the intestine, similar to that in the kidney, for L-lysine, L-ornithine, L-arginine, and L-cystine.

It seems rather surprising that a neutral amino acid (cystine) should share a transport system with three basic amino acids. In an attempt to discover some structural similarity between these four compounds molecular models were constructed. Of the many possible configurations one arrangement showed certain features common to all the compounds (Figure 62). Close apposition of the second amino group with the carboxyl group was possible in all cases. Perhaps this configuration is required for interaction with the carrier. The figure includes a new compound (a disulfide of cysteine and homocysteine) recently discovered in the urine of cystinuric individuals.[22a]

TRANSPORT SYSTEM FOR L-PROLINE, HYDROXY-L-PROLINE, SARCOSINE, DIMETHYLGLYCINE AND BETAINE

In the course of an investigation on the transport of neutral amino acids Hagihira et al.[235] found that betaine and N, N-dimethylglycine were actively transported by sacs of hamster intestine (see Figure 63 for structures). These compounds competed with one another but had no effect on the transport of neutral amino acids.

Furthermore, most neutral amino acids had relatively little effect on betaine transport. Two striking exceptions were L-proline and hydroxy-L-proline, which were extremely potent inhibitors of betaine transport. Furthermore, betaine inhibited L-proline transport. However, L-proline has a weak affinity for the neutral amino acid transport system and is partially inhibited by neutral amino acids.

One hypothesis which would explain the anomalous behavior of L-proline is that this amino acid shares two different transport systems,

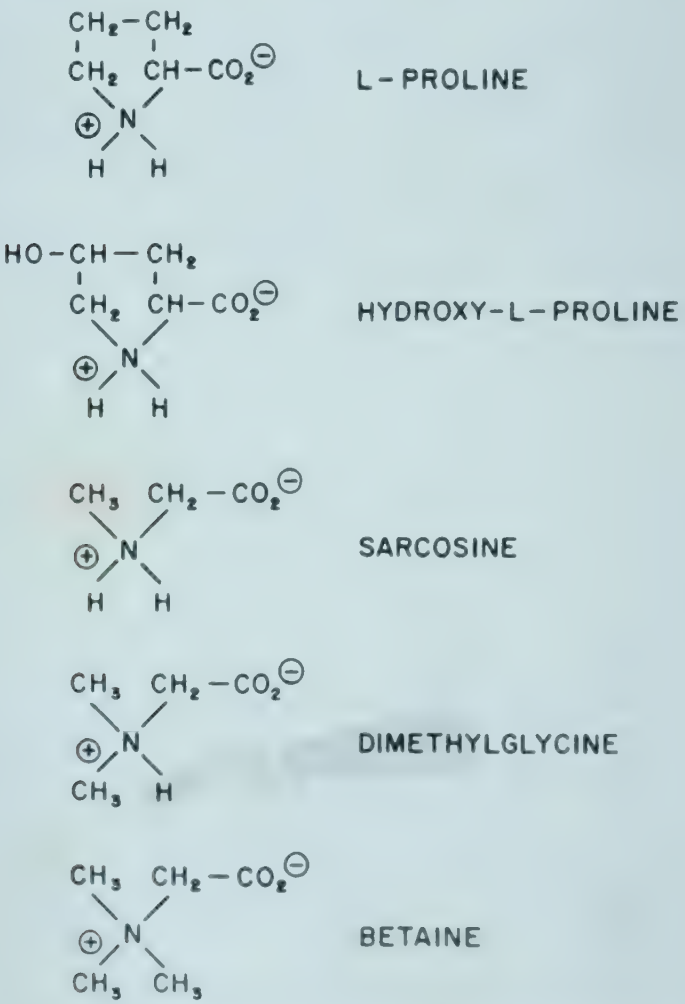

Figure 63. Proline, hydroxyproline, sarcosine, dimethylglycine, and betaine.

possessing a strong affinity for the betaine transport system and a weak affinity for the neutral transport system. It is believed that the separate transport system is normally utilized by proline and hydroxyproline as they would be effectively excluded from the neutral amino acid transport system by the many neutral amino acids always present in the small intestine.

TRANSAMINATION DURING ABSORPTION

Wiseman[79] incubated isolated rat intestine in the presence of either glutamic or aspartic acid and noted considerable loss of the amino acid from the medium. He postulated that these compounds might be transaminated in the epithelial cells. Matthews and Wiseman[48] then showed that, associated with the disappearance of glutamic acid, which occurred from both the mucosal and serosal sides of the intestine, there was an appearance of alanine on the serosal side. Incubation of rat intestine with aspartic acid resulted in a similar production of alanine plus a small amount of glutamic acid.

These results were then confirmed by Neame and Wiseman[54, 55] in anesthetized dogs, cats, and rabbits. They cannulated a mesenteric vein draining a loop of intestine *in vivo*. After introducing a solution of glutamic acid into the tied loop they collected the venous blood draining that loop and determined various amino acids. Figure 64 shows

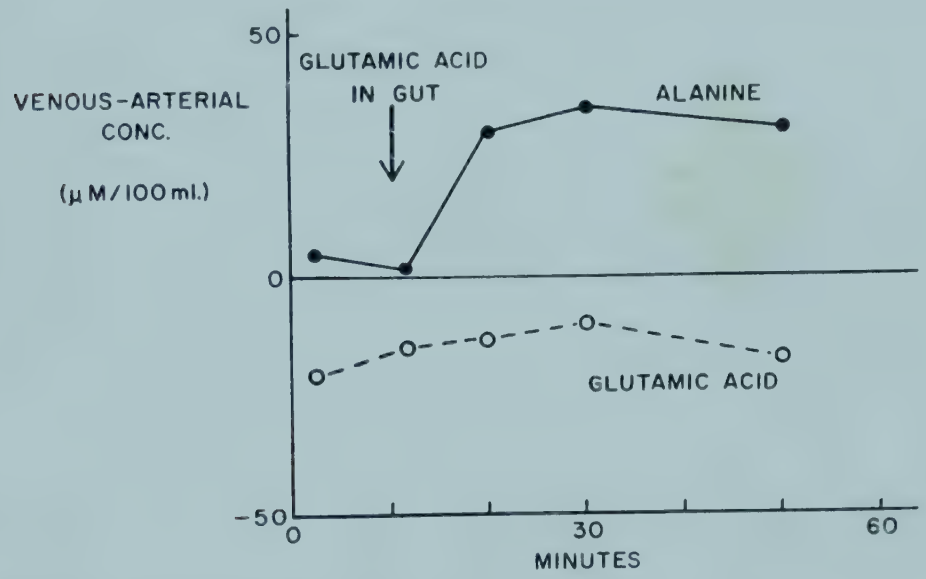

Figure 64. Production of alanine during glutamic acid absorption. Amino acid concentration was determined in an artery and a mesenteric vein of an anesthetized dog. The broken line indicates the difference in concentration of glutamic acid, the solid line indicates the difference in concentration of alanine and the arrow shows the time at which 10 ml. of 0.15 per cent glutamic acid solution was introduced into the lumen of the intestine. (Redrawn from Neame and Wiseman: J. Physiol., *135*:442, 1957.)

the results of one such experiment. During the control period the concentration of alanine was about the same in the artery and vein, while the concentration of glutamic acid was lower in the vein. Following the introduction of glutamic acid into the intestine the alanine concentration of the vein rose. These observations were confirmed in the cat and rabbit.

These results explain the previous observation of Dent and Schilling[16] that following casein feeding the concentration of glutamate in the portal blood was much lower than expected in view of the very high glutamate content of the fed protein. Neame and Wiseman point out[55] that on inspection of the chromatograms of Dent and Schilling[16] there is an unexpected increase in blood alanine.

The source of the keto acid required for the transamination reaction was investigated. There was no consistent change in the pyruvic acid or α-ketoglutarate concentration of the blood passing through the intestine during glutamate absorption. It was therefore presumed that glutamic acid transferred its amino group to pyruvate produced by the epithelial cells and that the resulting α-ketoglutarate is metabolized by the intestine. *In vitro*, however, some of the α-ketoglutarate spills out of the cells and can be recovered in the medium.[72]

There is an interesting aspect of dicarboxylic acid absorption which is, as yet, unexplored. Other dicarboxylic acids such as fumarate are very poorly absorbed by the intestine (see Chapter 12), presumably because they are too large to pass through water-filled channels of the membrane and they are insoluble in lipid because of their charge. It is to be expected, therefore, that glutamic and aspartic acids would diffuse across the epithelial cells only very slowly. Since the L-dicarboxylic amino acids are well absorbed it would seem reasonable to postulate some type of carrier-mediated transport mechanism for intestinal absorption of these acidic amino acids. Wiseman[79] showed that glutamic and aspartic acids were not actively transported by *in vitro* preparations of small intestine but it is possible that the extensive transamination might have obscured the process. Or, there may be some type of carrier-mediated transport independent of cell energy which "facilitates" the entrance of the substance into the cell. These possibilities should be investigated.

COMPARISON OF AMINO ACID TRANSPORT IN KIDNEY AND INTESTINE

There is now good evidence that the amino acid transport systems in the kidney and intestine are similar (Table 27). The evidence for the existence of separate neutral and "basic" amino acid "pumps" in the two organs is excellent. Not only is there evidence from studies of experi-

Table 27. Comparison of Amino Acid Transport by Kidney and Intestine

Amino Acid Transport System	Evidence for Existence of Transport System in:	
	Kidney	Intestine
Cystine Arginine Ornithine Lysine	Cystinuric defective in tubular resorption of these four amino acids.[15, 67] Competition with each other but little cross reaction with neutral amino acids.[8, 71, 61]	Cystinuric defective in absorption of lysine and ornithine.[49] Competition with each other but little cross reaction with neutral amino acids.[23a]
Neutral Amino Acids	In Hartnup's disease defect in resorption of neutral amino acids (except proline and hydroxyproline).[5, 33] Competition with each other.[6, 37, 71]	In Hartnup's disease defect in absorption of tryptophan.[50, 64] Competition with each other.[60, 3, 58, 34, 24, 53, 20, 66, 43, 44]
Proline Hydroxyproline (Sarcosine) (Dimethylglycine) (Betaine)	Proline resorption normal in Hartnup's disease.[5] Proline competes with hydroxyproline (and glycine) but not with others.[63, 63a]	All compete with each other. Little cross reaction with neutral group (except proline which may share both transport systems).[23a, 23b]

mental animals but in each of these two cases there is a genetic defect in human subjects in which a single transport system appears to be absent from both kidney and small intestine. Patients with Hartnup's disease lack the neutral amino acid pump while cystinurics lack the pump for cystine, lysine, arginine, and ornithine.

The evidence for the presence of a proline-hydroxyproline transport system in the two organs is good but still incomplete. Scriver, Schafer, and Efron[63, 63a] have presented evidence for a separate transport system for proline, hydroxyproline and, perhaps, glycine. They point out that patients with Hartnup's disease fail to resorb all neutral amino acids with the striking exception of proline and hydroxyproline, whose transport is entirely unaffected in this condition. They have found that infusion of proline, hydroxyproline, or glycine into normal human subjects results in the appearance of the other two in the urine (with the exception of glycine, who infusion caused prolinuria in only one out of three cases). These amino acids apparently did not affect the neutral amino acid transport system as none of the other amino acids appeared in the urine in abnormal amounts. Further evidence for their hypothesis is provided by a few interesting human subjects who excrete large amounts of proline, hydroxyproline, and glycine in their urine.

Data on intestinal absorption suggest that a separate transport system is present for proline, hydroxyproline, sarcosine, N, N-dimethylglycine, and betaine. The available data suggest that the last three compounds

have a high affinity for their own transport system and little or no affinity for the neutral amino acid transport system. Proline and hydroxyproline, on the other hand, appear to have affinity for both systems. In the intestine all of the evidence, at present, suggests that glycine is transported exclusively by the neutral pump. There is no direct evidence that the renal pump for proline is the same as that in the intestine as no genetic defect has been studied in which the two organs have been compared. It would appear feasible to strengthen the hypothesis by a study of just such a genetic defect in man or animals.

As mentioned in the previous section, active transport of dicarboxylic acids by the intestine has not been demonstrated. This fact does not, however, exclude the possibility of a special transport system as extensive metabolism of these acids occurs within the epithelial cell and renders this investigation difficult. Lotspeich[46] has pointed out that data on renal transport of these compounds strongly suggest that extensive transamination occurs during resorption. The possibility that similar absorptive mechanisms for dicarboxylic amino acids are present in both organs should be considered.

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CHAPTER 6

Fluid and Electrolytes

INGESTED AND SECRETED FLUID AND ELECTROLYTES

Fluid and electrolyte absorption is one of the major physiological functions of the intestine. Impairment of this function even for a short time leads to a serious or even fatal loss of fluid and salt. This is in part a result of the very large amount of gastrointestinal secretions which must be constantly resorbed even in the absence of fluid intake. Consideration of the dietary intake alone gives an erroneous impression of the total load imposed on the intestinal tract. In man 7 or more liters of fluid are secreted into the gastrointestinal tract per day while only 1.5 liters are ingested.[13] As only about 150 ml. are lost in the feces, more than 8 liters of fluid are absorbed daily (Figure 65). Moderately large alterations in the intake, therefore, result in relatively small changes in total load. It has been calculated[13] that about 80 per cent of the NaCl load is provided by secretions and 50 per cent of K^+ and Ca^{++} from the same source. This means that each day a volume equal to twice the plasma volume, including many of its electrolytes, is secreted into the gut and resorbed.

A word should be said about the terms "secretion" and "absorption." The term "secretion" will be reserved for net movement of a substance from blood to lumen and "absorption" for the reverse movement. Brun

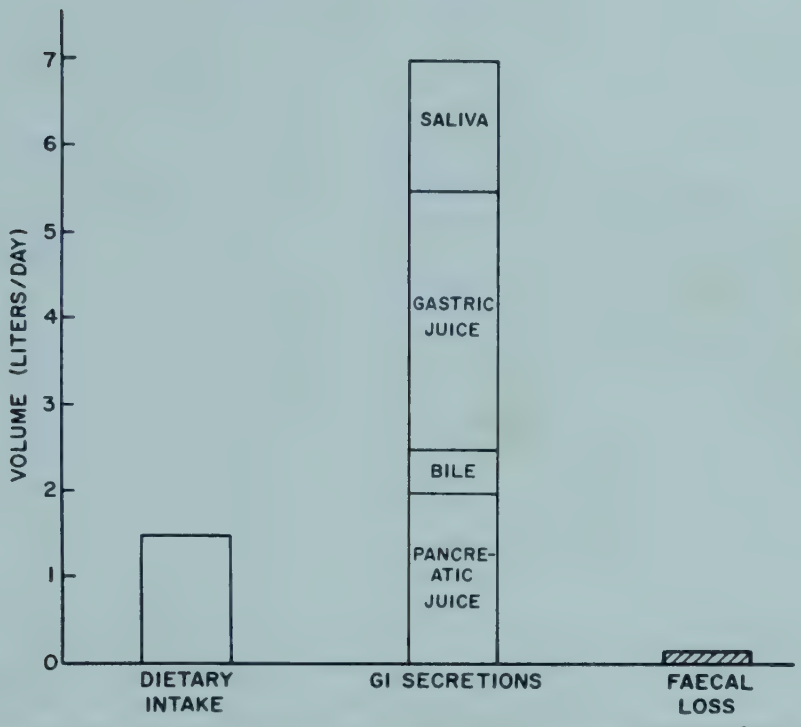

Figure 65. Fluid load imposed upon the intestine in man. (Drawn from the data of Carter et al.: Biochemistry in Relation to Medicine, Longmans, Green & Co.)

ner's glands of the duodenum, the only true glands in the small intestine, secrete an alkaline solution containing a high concentration of mucopolysaccharide.[33] The remaining portion of the small intestine produces a fluid, *succus entericus,* which is not a secretion in the same sense as salivary, gastric, or pancreatic secretion. This material produced by the small intestine contains mucus, desquamated epithelial cells, ions, and a small but variable amount of fluid. It can be obtained from a Thiry-Vella loop, but only with mechanical stimulation is an appreciable volume produced. Three types of cells with histologically recognizable secretory products have been identified: the goblet cell producing mucus, the argentaffine cell producing serotonin and possibly other substances, and Paneth cells whose secretory product is not known. The columnar epithelial cells are responsible for secretion of certain ions. Bicarbonate, iodide, and potassium ions are secreted into the lumen of the ileum and colon. When a variety of ionic changes occur simultaneously, the distinction between secretion and absorption may be confused. In an attempt to overcome this confusion other nomenclature has been suggested by Code.[16]

LOCATION

The stomach is not an important site of absorption of fluid or electrolytes. Although a flux of water occurs across the gastric mucosa in both

directions[82] there is little net absorption. Code and his collaborators[17, 50] have found that little net fluid absorption occurs in the duodenum although the flux rates for water and Na^+ are extremely rapid. They conclude that the duodenum fulfils the function of equilibrating the contents of the lumen with blood especially with regard to tonicity. Borgström et al.[8] have shown that in man the upper half of the small intestine is the major site of fluid absorption. They fed a solution of a nonabsorbable "marker" substance (polyethyleneglycol) which was diluted in the stomach and duodenum by secretions but returned to its original concentrations in the latter portion of the jejunum due to resorption of this fluid. In the anesthetized rat the jejunum absorbed almost twice as much Na^+ and water as the ileum.[70] Similar differences were found in the hamster intestine *in vitro*.[101] Berger et al.[6] found the colon considerably less active in NaCl absorption than the small intestine.

MECHANISM OF ABSORPTION OF SODIUM CHLORIDE AND WATER

(Active Transport vs. Passive Diffusion)

The study of fluid and electrolyte absorption has consumed the energies of many famous physiologists for over a hundred years and has been the source of numerous controversies, some of which are still unresolved. The experimental and theoretical problems are immense and recognition of some of the complexities has come only in the past few years. A few of these theoretical and practical problems are the following: differing behavior of anion and cation in a given salt, potential differences across membranes, colloid osmotic and Donnan effects, influence of solvent drag, and the differing behavior of weak and strong electrolytes. With such an array of complicating factors to consider it is little wonder that progress in this field has been slow.

Although a complete description of the events involved in salt absorption requires consideration of the electrical potential, this is not to say that valuable information cannot be obtained without it. The absorption of sodium chloride from the lumen of the intestine into the blood against an appreciable chemical gradient requires the expenditure of energy. This energy may be necessary for the active transport of the sodium ion, the chloride ion, or both. To distinguish between these possibilities one requires considerably more information, including data on potential difference and unidirectional flux rates for both ion species. Further complications arise because of the type of pore through which the ion passes and direction and magnitude of bulk flow of water. Considerable advance has been made in recent years in the evaluation of

these various factors as they apply to salt absorption in the gastrointestinal tract.

Historical

Sixty years ago a heated controversy raged between the proponents of diffusion as the primary force in intestinal absorption and those who believed that some "vital force" or "physiological activity" was responsible. One of the classical experiments supporting the latter view was performed by Reid in 1892.[76] He demonstrated net fluid movement across rabbit intestine *in vitro* when both sides were bathed in an identical saline solution (Figure 66). Since all osmotic forces were eliminated as the driving force for absorption, Reid concluded that some unknown factor associated with living cells was responsible for fluid movement. Cohnheim, independently, began studies on the isolated intestine of dog, cat, rabbit,[18] and octopus.[19] His extensive series of studies confirmed and extended Reid's observations. At the same time Heidenhain[48] was studying salt absorption *in vivo* and in 1894 showed that sodium chloride in hypotonic saline solutions was absorbed from the intestine, contrary to the laws of simple diffusion. The *in vitro* experiments were criticized as "unphysiological" and the *in vivo* experiments were ignored or dis-

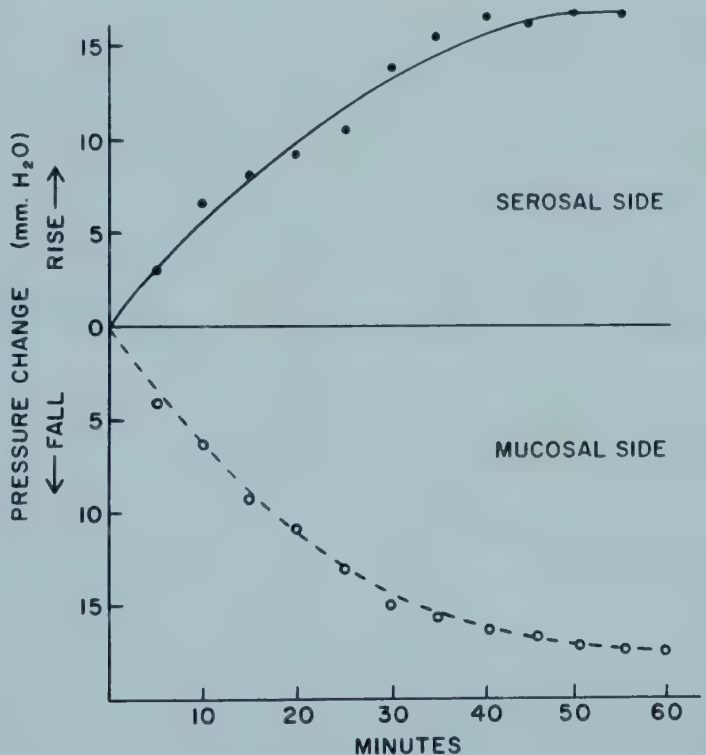

Figure 66. Movement of fluid across the isolated intestine. A segment of rabbit ileum was placed as a diaphragm between two compartments, each containing saline. A water manometer was attached to each compartment. During incubation pressure rose on the serosal side and fell on the mucosal side. (Drawn from the data of Reid: Brit. M.J., p. 1133, 1892.)

counted by the opposition, whose experiments emphasized the role of diffusion in membrane phenomena in general and the small intestine in particular.

In 1919 Goldschmidt and Dayton[36-40] published a series of five important papers on intestinal absorption of fluid and electrolytes from the dog colon. They showed very clearly that both water and salt could move across the intestine in either direction by diffusion in response to concentration gradients. Goldschmidt ended his 1921 review[35] on intestinal absorption by emphasizing the importance of diffusion and belittling the concept of "vital cell activity."

Curiously enough, Goldschmidt in the same series of papers[36-40] had clearly shown two phenomena which could not be explained by diffusion: first, the movement of salt from lumen to blood against a concentration gradient (in confirmation of Heidenhain), and, second, absorption of chloride against a very high concentration gradient when mixtures of Na_2SO_4 and NaCl were added to the gut lumen. In spite of this evidence of his own he could not bring himself to accept the possibility that aside from osmosis there were other important processes which could not be explained by the known laws of diffusion.

Visscher and his collaborators were largely responsible for the modern views of salt absorption from the intestine.[53, 54, 92-96] They studied NaCl absorption in the presence of one of the poorly absorbed salts, Na_2SO_4 or $MgSO_4$. One of their early experiments is shown in Figure 67. In the presence of Na_2SO_4 the chloride concentration fell to a very low level. Although the Donnan effect of the slowly diffusible $SO_4^{=}$ would

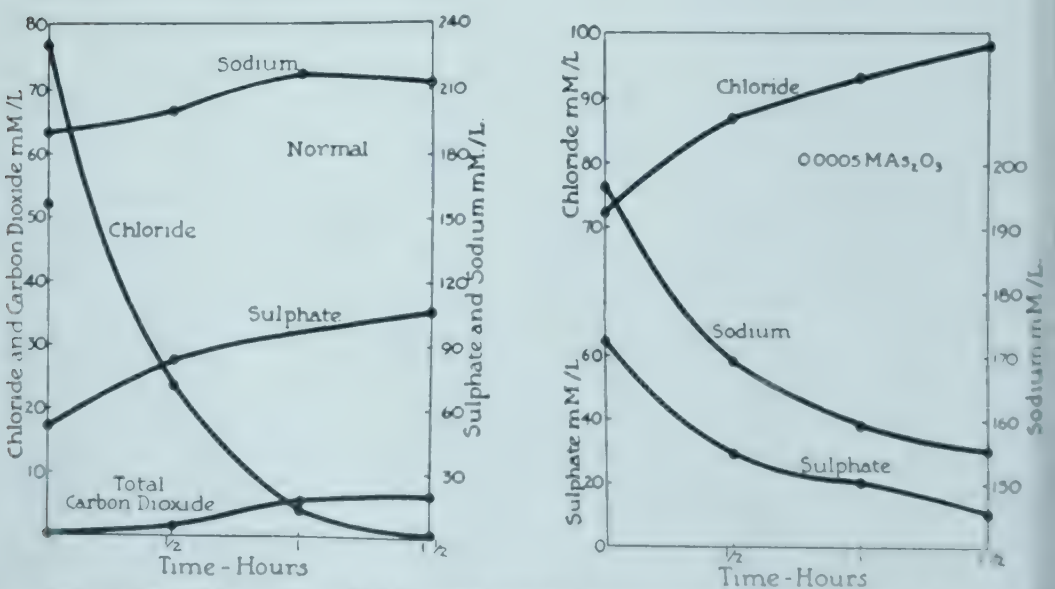

Figure 67. Absorption of chloride against a concentration gradient and its inhibition by arsenite. A mixture of sodium chloride and sodium sulfate was introduced into the ileum of an anesthetized dog. Ingraham and Visscher, Am. J. Physiol., 114, 1936.

Table 28. Absorption of NaCl and H_2O from Serum Placed in the Intestine of the Dog

	INTESTINAL CONTENT							BLOOD OSMOTIC PRESSURE
	VOLUME ABSORBED (%)	Na^+		Cl^-		OSMOTIC PRESSURE		
		INITIAL (mM.)	FINAL (mM.)	INITIAL (mM.)	FINAL (mM.)	INITIAL (mOsmol.)	FINAL (mOsmol.)	FINAL (mOsmol.)
Control	44	154	144	110	74	159	144	158
Poisoned by $HgCl_2$	−135*	148	148	110	106	159	156	158

*Secretion of fluid into the lumen.

Ten to 50 ml. of dog serum placed in washed ileal loops of anesthetized dogs for 40 minutes. Average values taken from Visscher, Roepke, and Lifson: Am. J. Physiol., *144*:457, 1945.

tend to lower the Cl^- concentration it would not account for some of the extremely low Cl^- values observed. Furthermore, from other experiments, it was clear the Na^+ also was absorbed against a chemical gradient which could not be explained by a Donnan effect. In the presence of $HgCl_2$ the absorption of Cl^- did not occur.

One serious criticism of many experiments on absorption in the living animal is that the solution which bathes one side of the epithelial cell (extracellular fluid or blood plasma) offers too many variables, including diffusible and nondiffusible anions, cations, and neutral molecules. In most experiments it was not possible to eliminate the possibility that the colloid osmotic pressure of the plasma was responsible for the absorption of fluid and electrolytes from the lumen of the intestine. A crucial experiment was the demonstration of fluid and salt absorption from serum placed in the intestine. Although this experiment was first performed in 1869 by Voit and Bauer,[97] the most complete experiment was reported by Visscher, Roepke and Lifson[95] (Table 28). Sodium chloride and water were absorbed from the intestinal lumen and the protein concentration increased. Although this type of experiment has been severely criticized because of possible protein hydrolysis in the gut lumen, such an occurrence would tend to make the osmotic pressure higher, which in turn would cause movement of fluid from blood to lumen, the opposite of what is actually observed.

Ion Fluxes and Potential Measurements

Although Na^+ transport occurs throughout the entire length of the small and large intestine, the colon provides the most favorable conditions for its demonstration experimentally. Ussing and Andersen[90] reported the first careful study of the relationship between the electrical properties of the intestine and the movement of Na^+. Using the elegant

Table 29. Sodium Transport in Large Intestine of the Toad

PERIOD NO.	TIME HR.	Na^+ INFLUX (LUMEN → BLOOD) μEq./hr./cm^2	Na^+ OUTFLUX (BLOOD → LUMEN) μEq./hr./cm^2	NET FLUX μEq./hr./cm^2	ELECTRICAL CURRENT IN μEq./hr./cm^2	ELECTRICAL CURRENT IN μAMPS
1	2	2.10	0.723	1.38	1.75	23.5
2	2	2.50	1.033	1.47	1.36	18.3
1	2	1.34	0.47	0.87	1.79	24.0
2	2	2.46	0.70	1.76	1.53	20.6

Tissue was placed as a diaphragm between two fluid compartments from which samples could be withdrawn. Current was measured in the short-circuited preparation of Ussing and Zerahn.[91] Taken from Ussing and Andersen: Third International Congress of Biochemistry, Brussels, 1955.

short-circuited method of Ussing and Zerahn[91] they showed that most of the short-circuit current was due to Na^+ transport. Table 29 shows the results of experiments with toad colon. Similar results were obtained with guinea pig cecum. The net transport of Na^+ was generally larger than the current output. This would indicate either a transport of a cation in the opposite direction (perhaps K^+) or transport of an anion in the same direction (perhaps HCO_3^-). Neurohypophyseal extracts cause an increase in short-circuit current and in net Na^+ transfer.

Cooperstein and Hogben[21] have recently studied the isolated large intestine of the bullfrog and found it to possess a spontaneous potential of about 45 mv. When both sides of the tissue were bathed with a Ringer solution containing bicarbonate and CO_2, measurements were made of flux rate for sodium and chloride as well as electrical parameters. Table 30 shows that the net movement of sodium of 2.25 μEq. cm.^{2} hr. occurred from mucosal to serosal side, a movement against an electrical gradient. Similar experiments were performed in the short-circuited frog colon (the data have been presented in Table 9, p. 56). The data clearly show the passive nature of the chloride movement. Sodium transport

Table 30. Simultaneous Measurement of Na^+ and Cl^- Fluxes at 45 mv. in Presence of HCO_3^- and CO_2

	M → S	S → M	NET
Sodium flux	3.39	1.14	2.25
Chloride flux	2.17	1.17	1.00
Current	−0.13	0.12	
Conductance, mmhos.	2.57	2.22	

M → S, mucosal to serosal flux; S → M, serosal to mucosal flux. Flux and current expressed as μEq./cm.^{2}/hr. Positive current is in the direction M → S and negative current S → M. Taken from Cooperstein and Hogben, J. Gen. Physiol., 42:461, 1959.

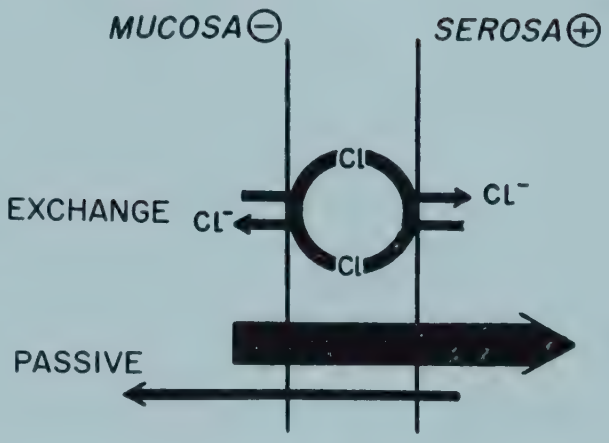

Figure 68. Chloride diffusion across frog large intestine. The carrier for exchange diffusion is designated as moving to and fro in the membrane. The large passive flux of chloride is in the direction of the electrical gradient. (Cooperstein and Hogben: J. Gen. Physiol., *42,* 1959.)

could account for most but not all of the short-circuit current. This suggests that in addition there was transport of another cation in the same direction or an anion in the opposite direction. Evidence was also obtained that bicarbonate facilitated sodium transport and, in addition, was necessary for the active transport of the other unidentified anion or cation. As the flux ratios for chloride were much less than that predicted by Ussing's equation[89] for passive diffusion it was concluded that the discrepancy could be attributed to exchange diffusion. A summary of the pathways for chloride is given in Figure 68.

Although the *in vivo* experiments are theoretically more complicated, having extracellular fluid and blood plasma on one side of the gut wall, they have provided important information which, by and large, is similar to that obtained with the *in vitro* systems. Thus, experiments on the colon *in vivo* of both rat[23] and dog[20] suggest active transport of sodium and passive diffusion of chloride. Cooperstein and Brockman[20] have provided evidence that during periods of secretion the dog colon may secrete bicarbonate ion against both an electrical and a chemical gradient.

The question of which ions are actively transported across the epithelium of the small intestine is somewhat more difficult than that in the case of the colon. Absorption rates for Na^+ and Cl^- are rapid in both the jejunum and ileum while the potential difference across the membrane is extremely small. Despite the experimental difficulties, Curran and Solomon[24] have succeeded in providing convincing evidence for the presence of active transport systems for both Na^+ and Cl^- in the small intestine. Although *in vitro* preparations appear to be somewhat more difficult to study in this regard[14, 15, 22] considerable additional data has been obtained. Tidball[87] has found that under special conditions of drug stimulation active transport of Cl^- from blood to lumen may be demonstrated. Secretion of iodide has also been demonstrated.[1-3, 72]

It is interesting to compare the different anatomical locations along the gastrointestinal tract and their ability to transport Na^+ and Cl^-

(Table 31). The stomach actively transports the Cl^- ion from blood to lumen while Na^+ movement is largely passive.[51] Both jejunum and ileum can clearly absorb NaCl against a concentration gradient, indicating transport of Na^+, Cl^-, or both. The unequivocal demonstration of one of these three possibilities in the jejunum and ileum has been difficult because of the extremely low electrical resistance of the tissue. The electrical potential difference is probably close to zero and the flux rates of ions in the two directions are very fast. Curran and Solomon suggest that both ions are actively transported in rat ileum.[24] Experiments with colon show clearly that Na^+ is actively transported while Cl^- moves passively. Chloride movement may have a component of either exchange diffusion[21] or solvent drag.[20]

Table 31. Movement of Na^+ and Cl^- across Different Regions of the Gastrointestinal Tract

REGION	SODIUM	CHLORIDE	pH
Stomach (secretion)	Largely passive	Active	Acid
Jejunum	Active??	Active??	Acid
Ileum	Probably active	Probably active	Alkaline
Colon	Active	Passive	Alkaline

Water Absorption

It has been known for many years that a hypertonic solution placed in the lumen of the intestine became diluted by a movement of water from blood to lumen and conversely hypotonic solutions in the lumen led to water movement in the opposite direction.[38] With iso-osmotic solutions in the lumen there was either no change in osmotic pressure during absorption or the solution became slightly hypotonic.[95, 94] Under these conditions, then, the solution being absorbed is iso-osmotic with blood or slightly hypertonic. These data are entirely consistent with the view that water moves in response to osmotic gradients set up either by adding an anisotonic solution to the gut lumen or by gradients produced by absorption of salt from an isotonic solution. This and other evidence[15, 22-24, 52] strongly suggests that the movement of water is secondary to the transport of solute (usually NaCl).

A few experimental situations have been discovered in which there is an apparently anomalous movement of fluid against an osmotic gradient both *in vivo*[37, 88] and *in vitro*.[31, 32, 71] Furthermore, hydrostatic pressure against the epithelial surface of rat intestine *in vitro* does not produce the expected movement of fluid.[32, 86] Active water transport has been suggested by some investigators[32, 34, 86, 93] to account for these results. A logical explanation without invoking a primary active water transport has been suggested by Curran.[22]

According to his hypothesis the *in vitro* intestine possesses two porous structures in series, a thin membrane with small pores (cell membrane) and a much thicker membrane with large pores (submucosal and muscle layers). Solute (usually NaCl) is actively transported across the thin membrane, which produces a local osmotic gradient, and fluid moves from lumen to cell across the thin membrane. Although there is a tendency for water to move from serosal to mucosal side across the thick membrane, such a process through the 200 to 500 microns of tissue is exceedingly slow. The movement of fluid across the thin membrane produces a hydrostatic pressure within the tissue and fluid passes across the thicker portion of the tissue due to the hydrostatic pressure gradient (bulk flow). It is quite reasonable, therefore, that under *in vitro* conditions the bulk flow of fluid from mucosal to serosal sides by solute transport effectively inhibits a tendency for diffusion in the opposite direction because of an adverse osmotic pressure difference on the two sides. The bulk flow hypothesis has been further strengthened by the observation of Lee[56a] that fluid moves across the *in vitro* rat intestine into the lymphatic and blood vascular channels.

There has been a discussion in the recent literature[32, 23] concerning the role of glucose in water absorption. This has arisen from the observation that rat intestine *in vitro* requires the presence of glucose for fluid absorption.[31, 32, 58, 71, 84-86] Lifson and Parsons[58] showed that this effect was a nutritive one as the presence of glucose on the serosal side in high enough concentration stimulated fluid absorption. It is important to remember that *in vivo* such additions of glucose to the solution in the lumen is without effect,[61] although there is, of course, always glucose in the blood. Furthermore, Wilson[101] has shown that *in vitro* preparations of hamster intestine do not require glucose in the incubation medium for ion and water absorption. It is concluded that the only effect of glucose is an indirect one on the nutrition of the cell and furthermore only occurs with *in vitro* preparations of rat intestine.

Alterations in Salt and Water Absorption

Physiologists have always assumed that intestinal motility had a profound effect on intestinal absorption although quantitative data to support this view had been meager. It has long been appreciated that violent increased motility meant that many substances were not in the small and large gut long enough to be completely absorbed. Recently, Higgins, Code, and Orvis[49] have shown with quantitative methods that decreased intestinal motility produced by methantheline bromide produced a decrease in Na^+ absorption.

Mercurials which are known to affect Na^+ resorption by renal

Table 32. Effect of Whole-Body Irradiation on Sodium Transport by the Intestine

HOURS AFTER IRRADIATION	NET SODIUM FLUX μEq./hr./cm.
0	8.5
6	4.0
13	1.1
21	−2.9
67	−6.0

Intestine taken from irradiated rats was placed in an *in vitro* apparatus and sodium and water movement measured. Taken from Curran, Webster, and Hovsepian: Radiation Res., *13*:369, 1960.

tubules also inhibit Na^+ transport by the intestine in experimental animals[7, 92] and in man.[42]

X-irradiation of the small intestine has been studied in some detail by Quastler.[74] Death as a result of whole-body irradiation of 100 to 10,000 r, which occurs in three to five days in the rat is mainly due to damage to the intestinal epithelium. Curran, Webster, and Hovsepian[25] have studied the epithelial defect in Na^+ absorption by measuring ion movement, *in vitro*, with intestine removed from irradiated rats. Table 32 shows that within six hours of irradiation net Na^+ absorption was significantly reduced and after a few days there was a reversal of ion movement (secretion into the lumen).

OTHER MONOVALENT IONS

pH Changes Across the Gut Wall (HCO_3^- and H^+)

Ions other than sodium and chloride must be considered in any complete description of intestinal absorption and secretion. The pH gradients across the intestinal epithelium can be considerable. In most animals jejunal contents are more acid than blood while ileal contents are more alkaline. These gradients must be a result of the active transport of one or more ions, the most likely candidates being HCO_3^- and H^+.

The first careful study of acid-base changes across the wall of the intestine was carried out by deBeer, Johnston, and Wilson,[27] who collected secretions from Thiry-Vella loops of unanesthetized dogs. Table 33 shows that the jejunum secreted fluid with a pH and "total CO_2" lower than that of blood plasma, while the ileum and colon produced an alkaline solution with a high "total CO_2." Of particular interest is the comment that the calculated CO_2 tensions were often much higher than those found in blood. The low bicarbonate and high CO_2 tension was also found in human subjects by McGee and Hastings.[59] In these experiments the calculated CO_2 tension ranged between 60 mm. and 200 mm. In the

Table 33. pH AND HCO_3^- CONCENTRATION OF INTESTINAL SECRETIONS OF THE DOG

LOCATION	pH	"TOTAL CO_2" ($HCO_3^- + CO_2$)
		(mM.)
Jejunum	6.8	19*
Ileum	7.6	83
Colon	8.0	90

* "If one calculates the CO_2 tension from our data, one obtains, in some instances, values far higher than those obtained for blood."
Secretions from Thiry-Vella loops of unanesthetized dogs, unexposed to air during collection.
Taken from deBeer, Johnston, and Wilson: J. Biol. Chem., *108*:113, 1935.

jejunum the dissolved CO_2 was clearly not in equilibrium with the blood and these authors concluded that two secretions were produced, one acid and the other alkaline.

When various solutions are instilled into the lumen of the small intestine and then removed after different time intervals, characteristic pH and bicarbonate changes are observed. In the dog,[10, 54, 77, 78] rat,[77] and man,[11, 59] solutions placed in the jejunum become slightly acid compared to blood, and similar solutions in the lower ileum become alkaline. D'Agostino, Leadbetter and Schwartz[26] have studied bicarbonate secretion

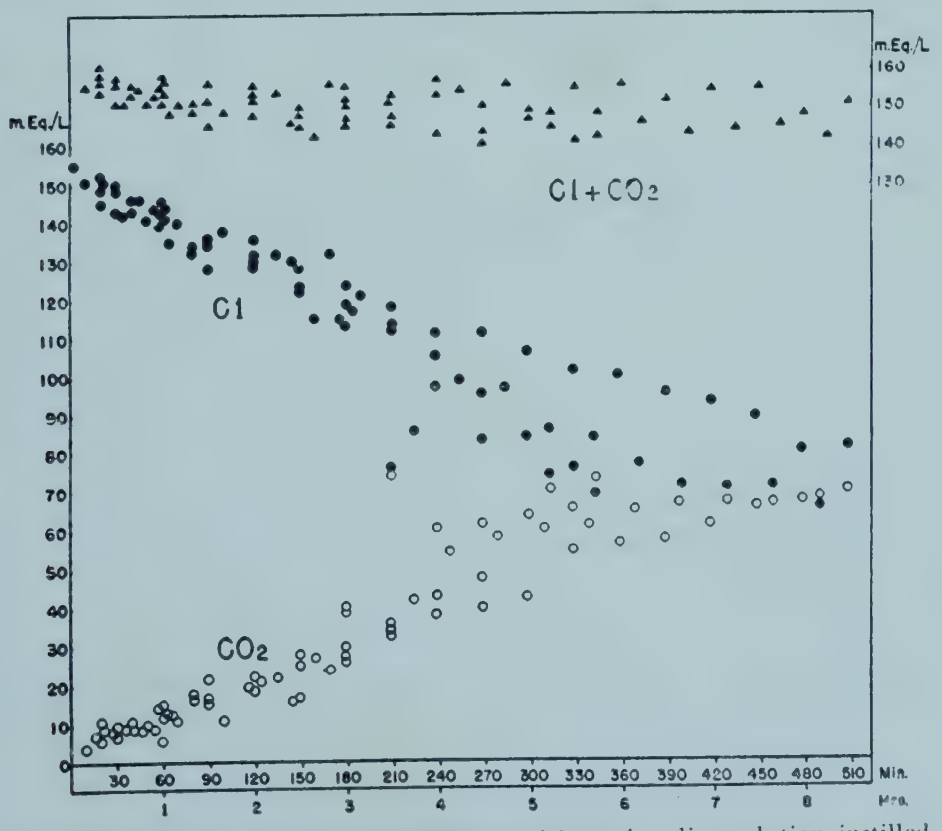

Figure 69. Changes in anion composition of isotonic saline solution instilled into the colon of the dog. (D'Agostino et al.: J. Clin. Invest., *32*, 1953.)

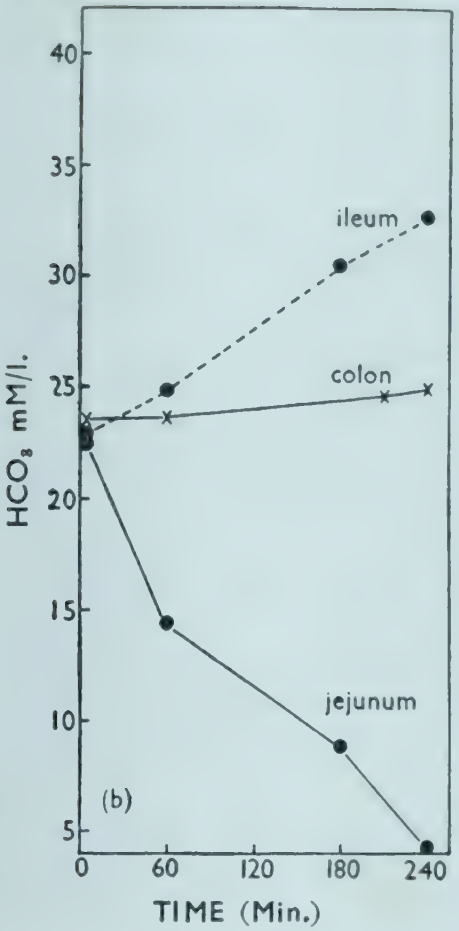

Figure 70. Changes in bicarbonate concentration during absorption of NaCl-$NaHCO_3$ solutions by rat intestine. Solution was recirculated through the lumen of the intestine of an anesthetized rat. (Parsons, Quart. J. Exper. Physiol., *41*, 1956.)

in the colon of the dog. Figure 69 shows the rise in bicarbonate and fall in Cl concentration of a NaCl solution placed in the colon.

The availability of newer methods has stimulated a reinvestigation of this problem. Wilson[100, 102] has shown that rat intestine *in vitro* develops a pH gradient across its wall, the lumen being acid in comparison with the serosal side. Parsons[70] made a careful study of the acid-base changes across rat intestine *in vivo*. Figure 70 shows that when a solution containing 25 mM. HCO_3 is placed in the jejunum, this ion was almost completely removed in four hours. In the ileum and colon the bicarbonate concentration rises under similar conditions. He also observed that the calculated H_2CO_3 concentration in the jejunum (1.97 mM.) was greater than that in either the ileum (1.70 mM.) or the colon (1.55 mM.).

Further studies were carried out on bicarbonate secretion in hamster ileum by Wilson and Kazyak.[102] When everted sacs of hamster ileum were incubated with Krebs-Henseleit bicarbonate-saline on both sides of the intestinal wall the pH and HCO_3 fell on the serosal side and rose on the mucosal side. The loss from the mucosal side could be largely ac

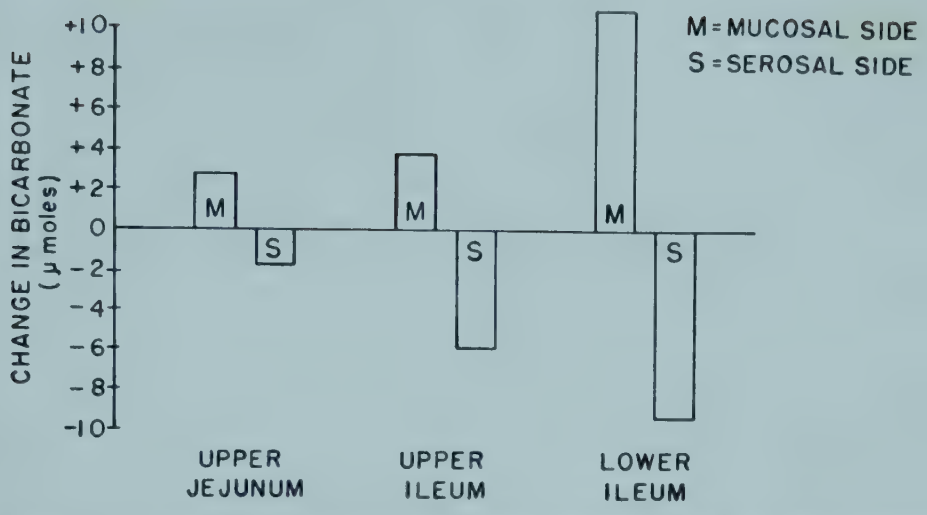

Figure 71. Bicarbonate secretion at different locations along the small intestine of the hamster. Net movement of bicarbonate across everted sacs of hamster intestine was estimated after 90 minutes incubation at 30°C. (Wilson and Kazyak: Biochim. et biophys. acta, *24*, 1957.)

counted for by the gain on the serosal side (Figure 71). Studies of fluid and ion movement suggested that the ileum was secreting an isotonic solution of Na^+ (or K^+) HCO_3^- from serosal to mucosal sides. During transport the CO_2 tension rose in the serosal solution. This observation was taken to indicate that the mechanism of transport involved the exchange of a hydrogen ion with some other cation across the serosal border of the epithelial cell (Figure 72).

From all of these studies it appears that when pH gradients are set up across the intestinal wall the more acid side has a high CO_2 tension. This is true for both the luminal solution during acid secretion by the jejunum *in vivo*[27, 59, 70] and the serosal solution during alkaline secretion

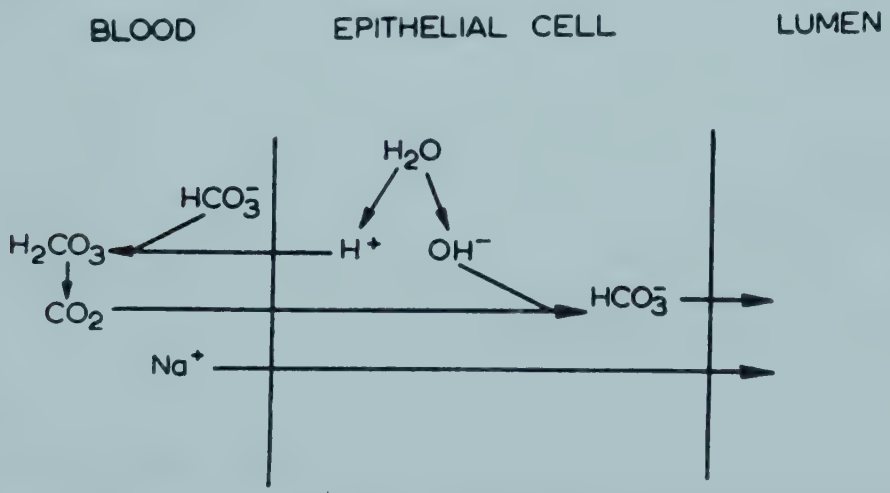

Figure 72. Postulated mechanism for bicarbonate secretion by hamster ileum. (Wilson and Kazyak, Biochim. et biophys. acta, *24*, 1957.)

by the ileum *in vitro*.[102] It seems reasonable to suppose that a similar mechanism is involved in both parts of the small intestine, one which involves the exchange or transport of hydrogen ion across one border of the epithelial cell. In the colon, for example, where the lumen is electrically positive with respect to the serosal side, the secretion of bicarbonate into the lumen[26] and the hypothetical movement of hydrogen ion into the blood would both occur against an electrical and chemical gradient.

Iodide

Pastan[72] discovered that the midportion of the rat small intestine secreted iodide from blood to lumen against a concentration gradient. This observation has been confirmed by the everted sac method by Acland et al.[1-3] Apparently the iodide secretion is not similar to that of the thyroid and salivary gland as it is unaffected by thiouracil and carbimazole.

DIVALENT CATIONS

The rate of intestinal absorption of divalent cations is extremely slow—many times slower than that for monovalent ions. It is fortunate, therefore, that the intestine is not called upon to absorb large amounts of such polyvalent ions. Apparently the absorption of even small amounts is difficult, and to survive the body has been forced to develop special mechanisms for their transport. The regulation of their transport is apparently quite complicated and at times the balance between need and supply is precarious. Calcium and ferrous ions are the two best known examples of divalent cations required by the animal body although other ions such as magnesium, manganese, and zinc are certainly required.

Calcium

In comparison with the total amount of sodium in the gut each day (about 1 mole) that for calcium is very much less (35 mM.) and only about half of that is absorbed. Although calcium is absorbed more than 50 times more slowly than sodium, this rate is considerably greater than that for the other divalent cations such as Fe^{++}, Zn^{++}, and Mn^{++}. In man about 1 gm. of calcium is secreted into the intestine from the salivary, gastric, and pancreatic secretions, and this, plus an amount equivalent to that lost in the urine, must be absorbed to maintain calcium balance. The view often expressed, even in recent texts, that calcium is actively excreted by the colon is apparently entirely erroneous. Nicolaysen,[65] for example, found that very little calcium was produced by a Thiry-Vella

loop of colon and this excretion was not influenced by either high calcium diet or intravenous injection of this ion. Furthermore, the same fecal calcium was found in animals with or without a colon.

A prominent feature of calcium absorption is the ability of an animal to increase its Ca^{++} absorptive capacity during periods of low intake.[66, 69] This "adaptation" to low intake was seen only in animals with adequate vitamin D intake and presumably is mediated directly or indirectly by this vitamin. The animal on a normal Ca^{++} diet but without adequate vitamin D will show a severe defect in calcium absorption. This requirement for vitamin D is the central theme in the story of calcium absorption and has been clearly demonstrated in a variety of animals (see recent reviews[68, 69]). That the vitamin directly affects the intestinal mucosa has recently been confirmed by transport studies with everted sacs of gut from normal rachitic rats.[29, 46, 47, 80, 81, 98] Figure 73 shows that the concentrating ability of the gut from deficient animals fell in a few days. As soon as one hour after replacement therapy, return toward normal had begun.[29]

The ability of the intestine to transport Ca^{++} across the gut wall against a concentration gradient is mainly confined to the upper portion of the small intestine in the rat, rabbit, guinea pig, and mouse. Curiously enough, the pattern is reversed in the golden hamster, the terminal ileum

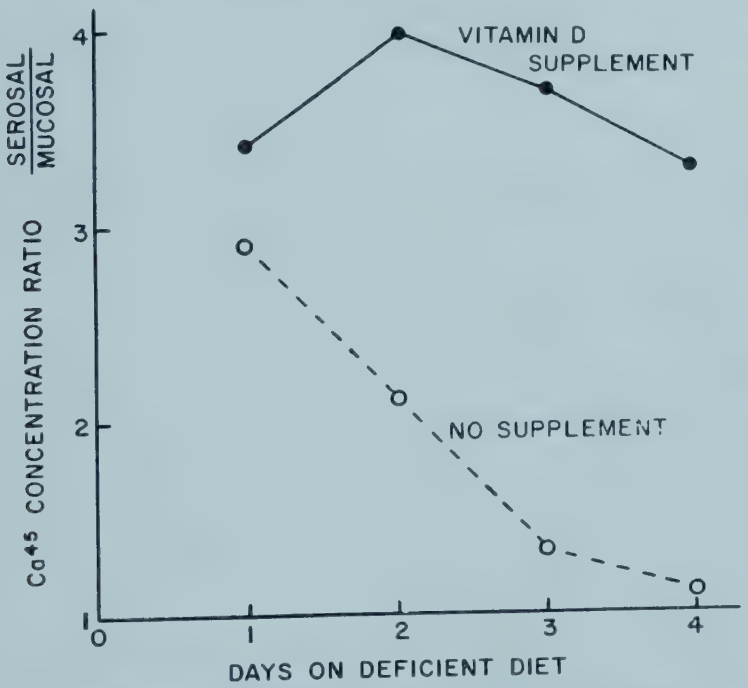

Figure 73. The effect of vitamin D on the intestinal transport of calcium. Rats were placed on a vitamin D–deficient diet or on a similar diet supplemented with vitamin D. Everted sacs of intestine from these animals were tested for their capacity to concentrate calcium.[45] (Redrawn from Schachter and Rosen: Am. J. Physiol., *196*:357, 1959.)

being most active. Most studies with the rat[67, 81, 99] and the chick[34] indicate that vitamin D affects the upper gut. *In vitro* studies of Schachter and Rosen[81] and others[99] clearly show effects on the duodenum.

DeLuca, Gran, and Steenbock[28] made the interesting observation that vitamin D inhibited citrate oxidation, resulting in an accumulation of citric acid in a variety of tissues. As citrate can form a soluble complex with calcium it is tempting to consider the possibility that citrate concentration within the gut or blood might regulate Ca^{++} absorption in some manner. *In vitro* studies have failed to provide any evidence to support this hypothesis, although it cannot be excluded according to the data available. Recently Rasmussen[75] has shown that parathyroidectomy reduced the ability of rat intestine to transport Ca^{++} (Table 34). He cites work on both rat and human to support his contention that the parathyroids may play an important role in Ca^{++} absorption.

Table 34. EFFECT OF PARATHYROIDECTOMY ON INTESTINAL TRANSPORT OF CALCIUM

CALCIUM CONC. (mM.)	TRANSPORT OF CALCIUM (FINAL SEROSAL CONC./FINAL MUCOSAL CONC.)	
	CONTROL	PARATHYROIDECTOMY
0.04	7.6	4.2
1.25	3.3	2.0

Sacs of rat intestine were incubated one hour with the indicated concentration of Ca^{++} on both sides of the intestinal wall. Tests on operated animals were carried out at least four hours after the operation. Modified from Rasmussen: Endocrinology, 65:517, 1959.

Iron

The daily dietary requirement for iron is very small. In humans, for example, less than 1 mg. needs to be absorbed by the gastrointestinal tract per day.[63] Although about 10 mg. are ingested daily, the body has difficulty in extracting the 10 per cent required. In women, who have a greater requirement for this ion, the extraction is not always entirely adequate. Recent reviews[12, 30, 56, 43] may be consulted for further details.

In the gastrointestinal tract iron exists in an equilibrium between the divalent and trivalent forms, the exact proportion of which is not known with certainty. From knowledge of permeability characteristics of other membranes one would expect that the trivalent species would penetrate the gut much more slowly than the divalent ion. It is quite clear from many studies in man and experimental animals that this is the case. Table 35 shows that both man and dog absorb the divalent ion faster than the trivalent.[64] It is probable that the ion species crossing the membrane is almost exclusively the divalent one even in the dog, as reduction of a certain amount of the trivalent to divalent certainly occurs. A factor which may inhibit iron absorption is the presence in the diet of large

amounts of phosphate or phytic acid which may precipitate ionized iron and make it unavailable for absorption.

Table 35. Absorption of the Reduced and Oxidized Forms of Iron

Dose of iron mg./kg. body weight	Fe^{++} absorbed / Fe^{+++} absorbed	
	Human	Dog
1	6.5 (2)	2.7 (9)
2	3.7 (3)	
4	4.6 (4)	

Numbers in parentheses indicate number of subjects. Both forms of iron were tested in the same animal in separate experiments. Taken from Moore, Dubach, Minnich, and Roberts; J. Clin. Invest., *23*:755, 1944.

A particularly interesting observation is the fact that anemic animals absorb dietary iron much more efficiently than do normal ones. Hahn et al.[44, 45] found that anemic dogs could absorb five to fifteen times as much iron as normal dogs. More iron is absorbed by humans in pregnancy and conditions in which iron stores are depleted.[5] These workers developed the hypothesis that the intestinal epithelium contained a receptor substance which would be saturated by iron in the lumen of the gut and could transport this iron into the blood. Granick[41] extended this idea by identifying the receptor substance with apoferritin in the membrane of the epithelial cell. The criticism of these theories is based mainly on the observation that the "saturation" of the carrier has not been confirmed. Smith and Pannacciulli,[83] for example, found that in human subjects a fairly constant percentage of the ingested dose was absorbed over a wide range of dosage (0.001 to 10mg.).

Brown and Justus[9] have recently studied Fe^{59} absorption with the everted sac method and found no evidence for factors other than diffusion during absorption. They observed that the $Fe^{59}SO_4$ uptake into the epithelium was greatest in the duodenum (*in vivo*) and declined gradually toward the low ileum. Dowell, Schachter and Schenker[29] reinvestigated the problem with *in vitro* preparations of rat duodenum and demonstrated absorption of Fe^{++} against a concentration gradient. This gradient was inhibited by substances which interfered with the metabolism of the cell (Table 36).

It is interesting to note that the duodenum, the only portion of the gut able to transport Fe^{++} against a concentration gradient,[29] is the portion of the gut containing most ferritin. Perhaps this is more than mere coincidence and ferritin is actually involved in the transport process. Further studies are required to elucidate the complete mechanism of this interesting process.

Table 36. Concentration Gradients of Fe^{59} Developed Across the Wall of Everted Sacs of Rat Gut

Conditions	Conc. on serosal side / Conc. on mucosal side
Aerobic control	8.2
Anaerobic	0.2
Aerobic, azide (0.003 M.)	0.6

Sacs of rat duodenum incubated with the same initial concentration of iron on both sides of the intestinal wall. Each value is the average of four experiments. Modified from Dowdle, Schachter, and Schenker; Am. J. Physiol., *198*:609, 1960.

Other Divalent Cations

Although Mg^{++} is essential for animal nutrition its absorption has not been investigated in any detail. This ion was tested by Schachter et al.[79] with *in vitro* preparations of rat intestine and they did not detect any transport against a concentration gradient.

Three other cations are probably required by humans in only milligram quantities: Zn^{++}, Mn^{++}, and Cu^{++}. It is possible that there may be unsuspected transport systems for these ions.

Wasserman[98] has presented evidence that sacs of rat ileum can secrete strontium ion from serosal to mucosal side. This ion is of interest because its metabolism within the body has some features common to Ca^{++}. The physiological significance of strontium secretion is not yet clear.

Other Anions

Sulfate: It has been known since the late nineteenth century that sodium and magnesium sulfates were poorly absorbed by the small intestine. The small intestine is not completely impermeable to the sulfate ion but absorption is so slow as to make it an effective saline cathartic.

Preliminary experiments with everted sacs of hamster intestine[103] indicate that sulfate can be absorbed by the jejunum and, in some experiments, secreted by the ileum against concentration gradients. This unexpected finding must be studied in more detail.

Phosphate: It is remarkable that so little work has been carried out on this interesting and important ion. Interest in this ion has centered mainly around its relation to calcium absorption. Large amounts of phosphate in the diet apparently depress calcium absorption,[57, 62] presumably because of the formation of calcium phosphate. This subject has been reviewed by Nicolaysen, Eeg-Larsen and Malm.[69]

McHardy and Parsons[60] have made a careful study of phosphate absorption from the rat intestine, *in vivo*. Net phosphate absorption increased with decreasing pH which may indicate that the monovalent

form is absorbed more readily than the divalent. The absorption rate was uninfluenced either by glucose in the lumen or by variation of the tonicity but was greatly diminished by low concentrations of sodium. Increasing the phosphate concentration in the lumen increased the absorption rate, but no evidence of any saturation phenomena was found.

In vitro studies of Jacobi, Rummel, and Pfleger[55, 73] have shown a high temperature coefficient ($Q_{10} = 5.3$) for phosphate absorption. Anaerobic conditions, dinitrophenol, and thyroxine increase the rate of phosphate movement from mucosal to serosal side. A relationship between phosphate absorption and aerobic glycolysis is suggested. The flux measurements of Asano[4] suggest the possibility that some type of carrier-mediated transport may be involved under certain conditions.

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CHAPTER 7

Triglycerides

Of the many types of lipid substances in the diet the triglyceride is quantitatively most important. Animal fat contains a large proportion of saturated fatty acids: stearic (C-18), palmitic (C-16) and the unsaturated (C-18) fatty acid, oleic acid. Only in butterfat are appreciable amounts of short-chain fatty acids found. In addition, animal and plant sources contain a very wide variety of other fatty acids with different degrees of unsaturation.

Virtually all of these naturally occurring fats and oils are absorbed and utilized by man and other animals. There is, however, variation in the extent to which animals absorb different fats. Ricinoleic acid, for example, the hydroxy mono-unsaturated fatty acid found in castor oil, is poorly absorbed in man but extensively absorbed and metabolized in rats. The digestibility of the different lipids in a variety of animals has been reviewed by Deuel.[67]

SITE OF ABSORPTION

Opinion differs as to the normal site of fat absorption and the section of the gastrointestinal tract with the greater capacity. Unfortunately much of the data are ambiguous. Kremmer, Linner, and Nelson[155] found that resection of the proximal intestine caused less of a defect in fat

absorption than removal of distal regions and concluded that the ileum was the site of absorption in the dog. Anatomical and functional changes are so marked following resections of the gut that such evidence is not altogether conclusive. In two laboratories the radioactivity in the gut wall after feeding I^{131}-triglycerides was measured and somewhat conflicting results were obtained. Turner[143, 144] found duodenum and jejunum most radioactive while Benson et al.[11] found the midgut most radioactive. Such studies do not measure intestinal absorption of fat directly but rather residual radioactivity in the mucosa.

A more direct approach to the question of the site of lipid absorption in man was made by Borgström, Dahlqvist, Lundh, and Sjövall.[45] They fed a measured quantity of lipid in a meal containing a nonabsorbable marker substance (polyethyleneglycol) and sampled intestinal contents at different levels of the intestine. They found a remarkably rapid absorption of lipid in the last portion of the duodenum and the proximal portion of the jejunum (Figure 74). Virtually all of the lipid had been absorbed at the level of the proximal ileum. This study gives a clear answer to the question of the normal site of absorption in man.

Johnston[93] found that the proximal jejunum was 10 to 20 times more active than the low ileum in the intracellular conversion of C^{14}-palmitate to triglyceride in everted sacs of hamster intestine. Similarly, Dawson and Isselbacher[62] found the jejunum of both the rat and human considerably

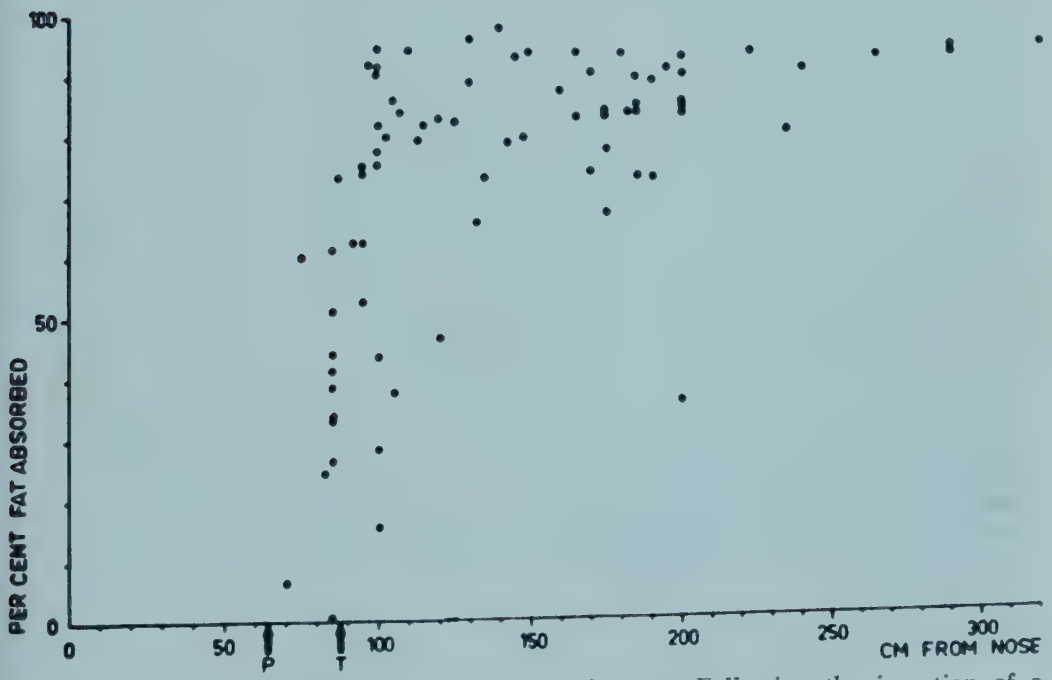

Figure 74. Site of triglyceride absorption in man. Following the ingestion of a test meal samples were withdrawn from the intestine and the percentage of absorption calculated. P = pylorus: T = ligament of Treitz (beginning of jejunum). (Borgström et al.: J. Clin. Invest., *36*, 1957.)

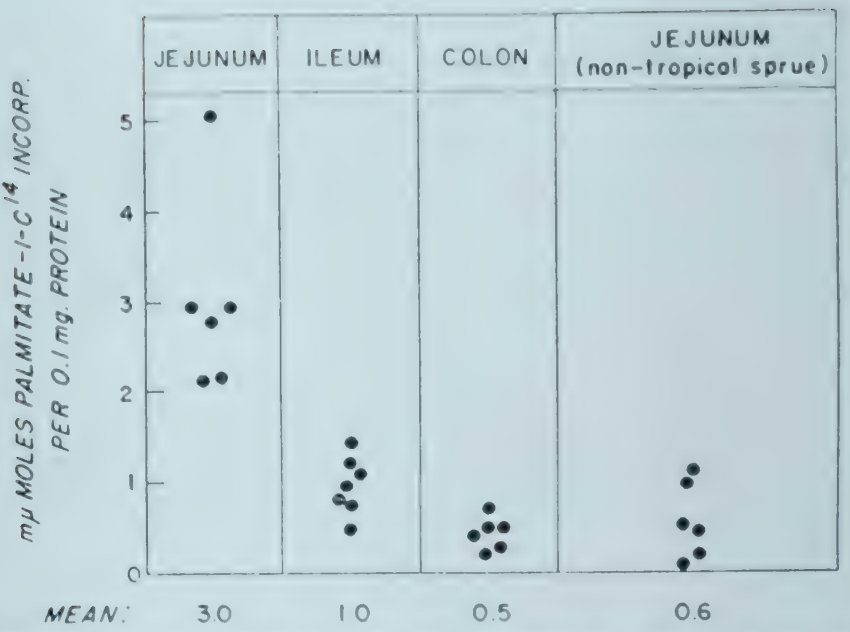

Figure 75. Conversion of fatty acid to triglyceride in different locations of the human intestine. Incorporation of C^{14} palmitic acid into glyceride by homogenates of intestinal tissue was measured. (Dawson and Isselbacher, J. Clin. Invest., *39*, 1960.)

more active than the ileum or colon in the conversion of fatty acid to triglyceride in homogenates of mucosa (Figure 75). Taken together, the evidence suggests that the upper small intestine is the main site of fat absorption and possesses the greatest capacity for triglyceride synthesis from fatty acids.

DIGESTION IN THE INTESTINAL LUMEN

Role of Lipase

The importance of pancreatic enzymes has been known since the classical observations of Claude Bernard.[17] In 1856 he noted that, following lipid feeding in the rabbit, cloudy lymphatics appeared in the duodenum 30 to 40 cm. below the entrance of the bile duct and immediately distal to the entrance of the pancreatic duct (Figure 76). This simple but elegant experiment suggested the presence of some substance in pancreatic juice essential for fat absorption.

Pancreatic lipase is apparently the most important enzyme in the digestion of lipid. In the stomach there is a rather weak lipase which, at least in man, acts mainly on tributyrin, having little effect on glycerides of long-chain fatty acids.[130] A small amount of gastric digestion of triglycerides *in vivo* (usually less than 10 per cent liberation of fatty acids) has been reported both in the rat[58] and in man.[85] In only a single case—hydrolysis of lard in the rat[58]—has extensive hydrolysis in the stom

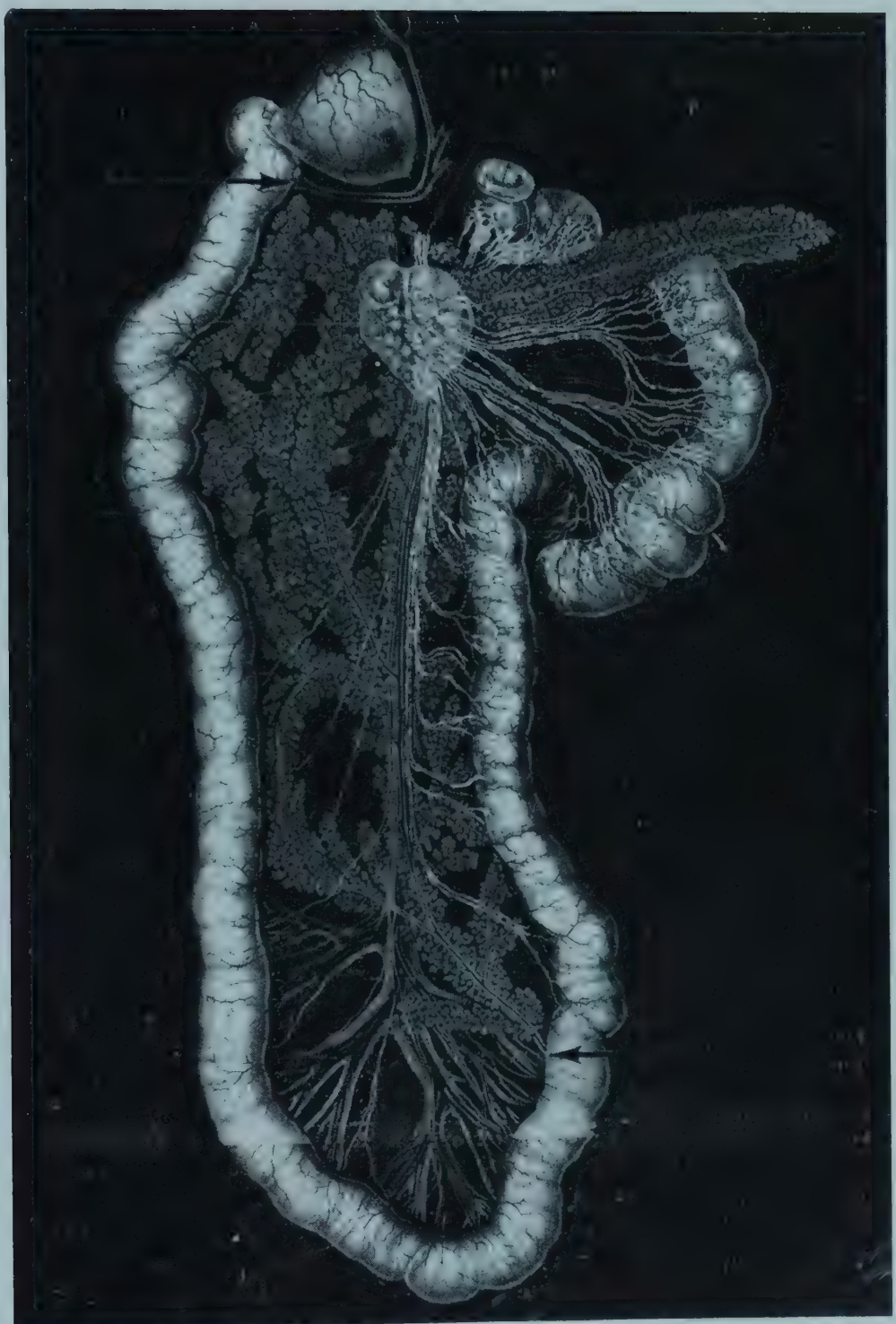

Figure 76. Lipid absorption in the duodenum of the rabbit. The bile duct enters the duodenum immediately distal to the pylorus (upper left); the pancreatic duct (lower right) enters the duodenum 30 to 40 cm. distal to the entrance of the bile duct. White lymphatics, indicating fat absorption, are most prominent distal to the entrance of the pancreatic duct. (Bernard, Compt. rend. Acad. sci., *43*, Suppl., 1856.)

ach been reported. In all *in vivo* experiments, especially the last one,[86] it is difficult to exclude the possibility of reflux of pancreatic juice into the stomach. Further studies including isolation and purification of gastric lipase are required to settle this question.

Recent evidence[141, 69] suggests that the small intestine possesses a lipase, distinct from pancreatic lipase, which may have an important physiological function in fat digestion. DiNella, Meng, and Park[69] state that, although the lipase activity per gram of small intestine is only about 5 per cent that of the pancreas, the large quantity of intestinal tissue may provide a significant total quantity of the enzyme. They further suggest that the fat digestion and absorption seen in the absence of pancreatic lipase may be largely due to intestinal lipase. Tidwell and Johnston[141] have found that everted sacs of hamster intestine liberate a lipase into the incubation medium which appears to show specificity for monoglycerides. The physiological role of this enzyme is at present under investigation.

The lack of either pancreatic lipase or bile salts produces a profound defect in the intestinal absorption of lipids. An early experiment published by Verzár[146] illustrates the effect of lipase and bile on olive oil absorption in the dog. Fat was administered with and without lipase (and/or bile salts) into a tied segment of dog intestine. After 24 hours the extent of fat absorption was estimated (see Table 37). In these experiments no olive oil absorption occurred in the absence of lipase. Loss of pancreatic secretions, either by pancreatectomy[83, 58, 145, 59] or by external pancreatic fistula[58] results in poor absorption of fat. Coffey, Mann, and Bollman,[58] for example, found that with either technique

Table 37. Effect of Bile Salts and Lipase on Fat Absorption

DOG NO.	INJECTED INTO INTESTINE	RECOVERED FROM INTESTINE AFTER 24 HRS. (GM.)	
		NEUTRAL FAT	FATTY ACIDS
a.	5.65 gm. olive oil in water emulsified with soap	5.50	0.05
b.	5.35 gm. olive oil in water + 5 ml. lipase	3.08	2.34
c.	5.27 gm. olive oil in water + 20% taurocholate	5.08	0.06
d.	5.52 gm. olive oil in water + 5 ml. lipase + 20% taurocholate	0.98	0.43

Dogs anesthetized with chloralose, common bile duct tied, gut washed out, ileum tied at ileo-cecal valve and gut filled with solution given in table. After 24 hrs. animals were killed and contents of gut washed out and analyzed.

Taken from Verzár: Ergebn. Physiol., *32*:447, 1931.

Table 38. Effect of Loss of Bile and Combined Loss of Bile and Pancreatic Secretions on Lipid Absorption in the Rat

DIET	TYPE OF ANIMAL	TOTAL FATTY ACIDS IN THORACIC DUCT (MG.)
Fat free	Normal	118
	Bile fistula	8.5
	Duodenal drainage*	8.0
Corn oil (0.6 ml./rat)	Normal	447
	Bile fistula	55
	Duodenal drainage*	7.6
Oleic acid (0.55 ml./rat)	Normal	398
	Bile fistula	165
	Duodenal drainage*	40

*Duodenal drainage prevented either bile or pancreatic juice from entering the small intestine. These animals were fed via duodenal tube. All other animals were fed by stomach tube. Taken from Kim and Bollman, A.M.A. Arch. Surg., *69*:247, 1954.

one half to three quarters of the fat in the diet was excreted in the feces. Studies of Kim and Bollman[102] in the rat suggest that a combined loss of pancreatic juice and bile produces a greater defect in lipid absorption than does the loss of bile alone (see Table 38).

Most workers agree that loss of pancreatic function in man results in considerable steatorrhea. In human subjects it is often so difficult to assess the extent of pancreatic insufficiency that some patients, presumed to have lost completely the pancreatic function, actually retain a moderate amount of lipase and show no defect in fat absorption. In cases where extreme care was taken to assay duodenal washings for the presence of lipase, good correlation was obtained between fat absorption and the presence of the enzyme. Beazell, Schmidt, and Ivy[10] studied four patients with loss of pancreatic acinar function due to chronic pancreatitis and found no pancreatic enzymes in the duodenum. Table 39 shows that during a four-day period on a diet of 11.2 gm. fat the daily fecal excretion was 40 to 84 per cent, while the same group fed pancreatin excreted

Table 39. Intestinal Absorption of Lipid in the Absence of Pancreatic Lipase (Human Subjects)

PATIENT NO.	FAT LOST IN FECES (PER CENT OF INGESTED FAT)	
	NO ADDITIONS	PANCREATIN
1	40	25
2	84	19
3	76	29
4	66	24

Normal fat excretion is about 7 per cent of intake. Each number in the table represents a four-day collection period. Taken from Beazell, Schmidt, and Ivy: J. A. M. A., *116*:2735, 1941.

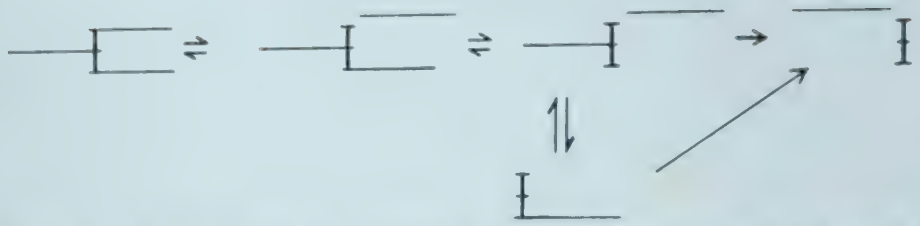

Figure 77. Hydrolysis of triglycerides by pancreatic lipase. (Modified from Borgström: In Chemistry of Lipides as Related to Atherosclerosis, Charles C Thomas.)

19 to 29 per cent. Similar results were obtained by others following resection of the pancreas.[49, 106]

Pancreatic lipase *in vivo* hydrolyzes triglycerides in a stepwise fashion to diglyceride, monoglyceride, and finally to glycerol and free fatty acid,[4, 77, 66] and all steps except the final one are reversible.[1, 65] This lipase does not hydrolyze the ester bonds of the triglyceride in a random manner but attacks the fatty acid attached to the α hydroxyl group of glycerol.[111, 40, 110] Figure 77 shows the course of this hydrolysis. Mattson and Beck[110] have shown that the 2-monoglyceride (β-glyceride) predominated in the products of hydrolysis but the 1-monoglyceride is also present in considerable amounts. It is not certain whether the enzyme has an absolute specificity for the 1 and 3 positions (in which case the 2-monoglyceride must be isomerized to the 1 position), or whether the 2 position of a diglyceride may be attacked. During hydrolysis of triglycerides by rat pancreatic lipase, added radioactive fatty acids exchange with fatty acids in the 1 and 3 positions of the triglyceride[41] indicating reversibility of at least the first reactions shown in Figure 77. Furthermore, when radioactive oleic acid was incubated with 1, 2-diolein, radioactive triolein was isolated. In addition, there is a preference for unsaturated fatty acids during hydrolysis.[110] The final step of monoglyceride conversion to free glycerol and fatty acid is physiologically irreversible as the glycerol is greatly diluted in the aqueous phase and then absorbed. Labeled glycerol fed with fatty acids does not result in glycerides containing the labeled glycerol.[52]

Karnovsky and Wolff[100] have tested the possibility that lipases might cleave symmetrical triglycerides stereospecifically to yield D-α,β-diglycerides. Under the conditions of their experiments no such specificity could be demonstrated.

The specificity of pancreatic lipase was studied by Balls and Matlack,[7] who found that single or double methyl substitutions on the α carbon atom of the alcohol in the ester prevented hydrolysis, whereas beta or gamma substitutions caused no effect. Tryding[142] has recently found that methyl substitution on the α and β carbon of the acid reduced the velocity of hydrolysis. For further details on this enzyme see reviews by Ammon and Jaarma[2] and Desnuelle.[64]

Effect of Bile in Fat Absorption

It has been known since 1890[115] that if bile is prevented from entering the small intestine fat in the stool is increased. In rats there is a severe defect in triglyceride absorption in the absence of bile.[18, 39, 119] Bernhard and Ritzel[18] found only 7 to 21 per cent absorption of deuterated fat fed to bile fistula rats. In the dog there is a definite impairment of fat absorption without bile,[137, 126, 147, 84] but it is not as extreme as in the case of the rat. Considerable quantities of fatty acids can be absorbed in the absence of bile,[119] which perhaps suggests a somewhat different mode of absorption. In bile fistula rats fed palmitic acid-C^{14}, only about one fourth of the absorbed fatty acids appeared in the chyle in comparison with over 90 per cent in the normal animal. Bile and sodium taurocholate enhance the absorption of sodium oleate from loops of dog intestine *in vivo*.[126, 147] Pessoa, Kim, and Ivy[119] found only a moderate defect in the fistula animals when they were fed corn oil or oleic acid but a severe loss of absorptive capacity when lard was fed. The results in animals have been confirmed in man by Shapiro et al.[131] An interesting human subject with a congenital absence of bile salts has been reported by Ross et al.[127] As shown in Figure 78, fecal loss can be minimized by feeding bile by mouth.

Extent of Hydrolysis

Knowledge of the extent of hydrolysis of triglycerides in the lumen of the intestine prior to absorption is of extreme importance in elucidat-

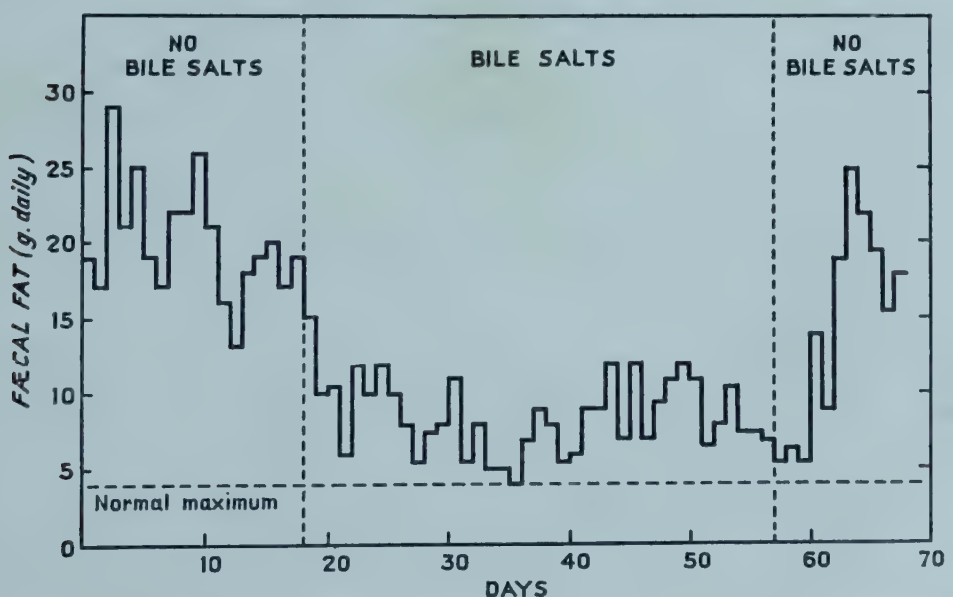

Figure 78. Effect of feeding bile salts to a human subject with a congenital absence of bile salts. Feeding of 10 gm. of bile daily dramatically reduced fecal fat. (Ross et al.: Lancet, *1*, 1955.)

ing the mechanism of transport across the epithelial cell. The dominant view for many years was that proposed by Pflüger in 1900[120-122] that complete hydrolysis of triglycerides occurred prior to absorption. This was first seriously challenged by Frazer, who presented evidence for only partial hydrolysis in the intestinal lumen.[77, 75]

In recent years somewhat more direct experiments have been possible with the use of isotopes. The results are so important that a brief consideration of the assumptions and experimental method are in order. The first and most important assumption is that free glycerol liberated by complete hydrolysis of triglyceride in the intestinal lumen or epithelial cell cannot be reutilized for triglyceride synthesis in the intestine. This assumption was proven to be correct by two types of evidence. First, labeled glycerol in the lumen plus fatty acids or triglycerides does not give rise to labeled triglycerides in the chyle or intestinal wall.[70, 71, 125, 19, 52, 79, 113, 80] Second, the intestine does not contain a glycerokinase[50, 149] (glyceride glycerol apparently comes from glycolysis[51, 56a]). An experiment performed by a number of workers[125, 19, 46, 25] was to feed a triglyceride with glycerol labeled in one fashion and the fatty acids labeled in another. Figure 79 shows a number of possible experimental results (assuming no utilization of free glycerol). Complete hydrolysis would lead to the loss of all of the glycerol, and the triglyceride isolated from the lymph would contain none of the labeled glycerol. With no hydrolysis, the ratio of labeled glycerol to fatty acids would be the same in the glycerides of the chyle as in that initially administered. Partial hydrolysis

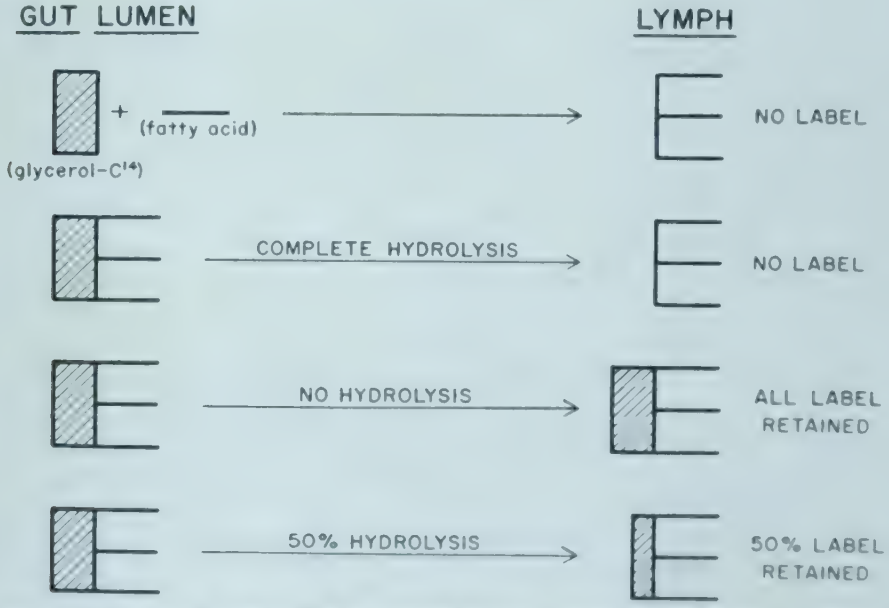

Figure 79. Possible fates of glyceride glycerol during absorption. Glycerol-C14 plus fatty acid does not give rise to labeled triglyceride in the lymph. Three possible consequences of feeding triglyceride containing glycerol-C14 are shown.

would result in partial loss of the glycerol. Four groups of workers[125, 19, 46, 25] have performed this experiment and have found 25 to 60 per cent complete hydrolysis of triglyceride molecules.

The labeling experiments described above, while providing data on the extent of complete hydrolysis, do not shed light on the chemical form of the glycerides that enter the cell (whether tri-, di-, or monoglyceride). There is evidence, however, that a considerable quantity of monoglyceride is formed.[125, 20, 25, 134, 124] A study of Blankenhorn and Ahrens[20] indicates that following triolein ingestion in man the composition of intestinal lipids was as follows: 58 to 60 per cent fatty acid, 13 to 17 per cent monoglyceride, 6 to 9 percent diglyceride and 4 to 6 per cent triglyceride.

After feeding doubly labeled glycerides Reiser et al.[125] and Blomstrand et al.[25] obtained evidence of monoglyceride absorption. Also, Skipski et al.[134] found that intestinal tissue contains monoglyceride and concluded that considerably intact monoglyceride is absorbed. Tidwell and Johnston[141] reinvestigated this problem with isolated tissues. Everted intestinal sacs of hamsters and weanling rats did not absorb diglycerides or triglycerides under conditions where monoglycerides were absorbed.

It is concluded from the above discussion that a considerable fraction of triglyceride is completely hydrolyzed to fatty acid and that most of the remaining fraction is partially hydrolyzed. The fact that appreciable quantities of monoglyceride are found in the lumen and that it is readily absorbed suggests that a major portion of ingested triglyceride is absorbed in the form of monoglyceride. Making this assumption and using estimates of complete hydrolysis it is possible to calculate the fraction of total glyceride–fatty acids liberated in the lumen during digestion. Such a calculation (Table 40) indicates that 75 per cent of the fatty acids are released in the intestinal lumen.

Table 40. Calculation of the Fraction of Total Fatty Acids Liberated from Triglyceride in the Intestine

1.	Assume:	25 per cent of triglycerides completely hydrolyzed (conservative estimate)
		TRIGLYCERIDE $\longrightarrow$ FATTY ACID + GLYCEROL
2.	Assume:	Remaining triglyceride completely converted to monoglyceride (no quantitative data available)
		TRIGLYCERIDE $\longrightarrow$ FATTY ACID + MONOGLYCERIDE
		50 per cent of original fatty acids liberated by this reaction
		75 per cent of total fatty acids liberated (assuming 1 and 2)

Origin of Fecal Fat

All animals excrete fat in their feces even when no fat has been taken in the diet. In man this amounts to about 2 gm. per day. The origin

of this lipid is in doubt and considerable controversy has existed over this question. Bergström and Borgström[14] have recently reviewed this question and find that the two main views on the origin of fecal fat are: (1) nonabsorbed dietary fat and (2) bacterial synthesis. They offer evidence that the first is the case in man (although the opinion is by no means unanimous[131]), while many workers favor the second hypothesis for other animals. The bile apparently contributes little to fecal fat as bile fistula animals always excrete considerably more, rather than less, lipid.[136]

In an attempt to explain this last observation another hypothesis has been advanced for the origin of fecal fat. According to this view the major portion of endogenous fat is derived from desquamated epithelium of the G.I. tract. This fat, like that of dietary origin, must be emulsified by bile salts, hydrolyzed by lipase, and absorbed into the lymphatics. If either bile, salts or lipase is missing, fat in the stool should increase during a fast. Kim and Bollman[102] found that 118 mg. of fat appeared in the lymph of a normal fasted animal in a 24 hour period while a similar animal with a biliary fistula had only 8.5 mg. in the chyle. This confirmed the previous observations in dogs that bile fistula animals excreted more lipid in the stool than normal dogs.[136, 119] Sperry and Angevine[138] calculated that 252 mg. of total lipid were present in the entire intestinal mucosa per kg. of dog. According to Leblond and Stevens[107] about 66 per cent of the entire small epithelium of the rat is desquamated per day. If this data is applicable to the dog, 166 mg. lipid would be excreted by this means per kg. per day. This agrees well with 142 mg. of lipid excreted in the bile fistula dog. Pessoa, Kim, and Ivy[119] conclude that desquamated epithelial cells constitute the major source of fecal fat.

The primary function of bile is presumably the emulsification of fat into droplets small enough to make adequate contact with lipase. "Solubilization" of complex mixtures of fatty acids and glycerides to molecular dimensions may also be very important for absorption (see review by Borgström[44]).

METABOLIC ALTERATIONS WITHIN THE EPITHELIAL CELL

Fate of Absorbed Fatty Acids

It has been known since 1884[114] that fatty acids fed to animals appeared in the thoracic duct as triglyceride. Sinclair,[138] in one of the first labeled experiments, fed elaidic acid and isolated from the lymph a triglyceride containing this unsaturated acid. The introduction by Bollman, Cain, and Grindlay[41] of methods for the collection of thoracic duct lymph in the unanesthetized rat, and the availability of radioactive iso-

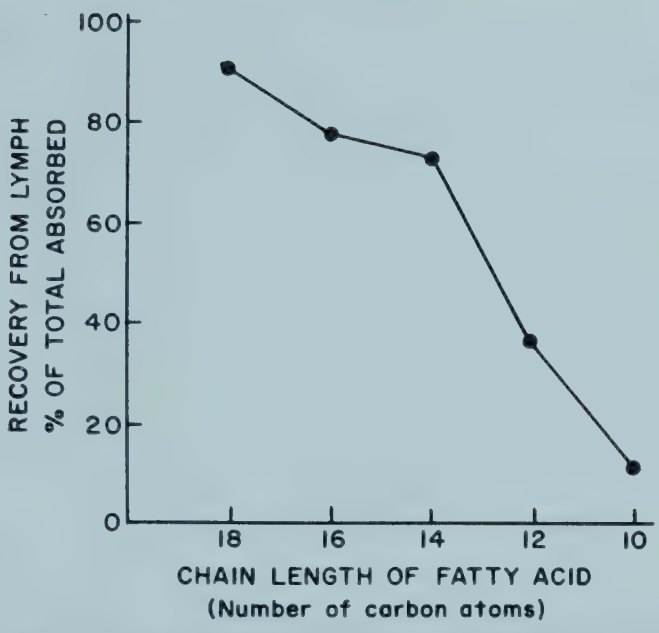

Figure 80. Effect of chain length on the route of absorption of fatty acids. (Drawn from the data of Bloom et al.: Am. J. Physiol., *166*:451, 1931; and Bloom et al.: J. Biol. Chem., *184*:1, 1950.)

topes stimulated renewed interest in the question of fatty acid absorption. In 1950 Bloom et al.[30] fed C^{14}-palmitic acid to rats and recovered 70 to 92 per cent of the absorbed acid in the lymphatics. In the same year Bergström et al.[15] fed C^{14}-stearic acid to cats and obtained much of the radioactivity in the chyle as triglyceride. There are now many studies which clearly indicate that fatty acids with more than ten carbon atoms, either saturated[13, 18, 20, 30, 15, 28, 53, 36-38, 104, 21, 23] or unsaturated,[13, 21] are converted to triglyceride by the epithelial cells and transferred to the lymph in that form. Figure 80 shows the fraction of the absorbed fatty acids of different chain length appearing in the lymph.

Occasionally a disease or an anomaly in a human subject provides the investigator with the opportunity of sampling chyle in an otherwise normal individual. In 1891 Munk and Rosenstein[116] had the opportunity of studying a 19 year old girl who had a lymphatic fistula draining most of the intestinal tract. After feeding fat to this subject, up to 60 per cent could be recovered in the chyle primarily in the form of triglyceride. Recent studies were made on patients with chylothorax and chyloperitoneum. They were fed fatty acids, and the quantity of acids appearing in the chyle measured.[72] One of the most carefully studied cases was a patient with chyluria[23a, 27] who was completely normal except for the fact that under certain conditions she excreted fat in the urine after a meal. A test diet containing 42 gm. of a mixture of corn oil and C^{13}-labeled oleic acid or triolein was fed and the urine collected. It was calculated from the total lipids and the isotope data that about 20 per cent of the chyle appeared in the urine. The distribution of lipids in the chyle is given

Table 41. Absorption of Triolein and Oleic Acid in a Human Subject

A. Distribution of fatty acids in chyle

FED	PER CENT OF TOTAL FATTY ACIDS IN CHYLE			
	GLYCERIDE	FATTY ACIDS	CHOLESTEROL ESTER	PHOSPHOLIPID
Triolein	92*	6.3	1.7	—
Oleic acid	79	7.9	3.0	9.9

*Includes phospholipids

B. Distribution of label (C^{13}) in chyle

FED	PER CENT OF TOTAL C^{13} IN CHYLE		
	GLYCERIDES PLUS CHOLESTEROL ESTERS	FATTY ACIDS	PHOSPHOLIPID
Triolein	99	1	—
Oleic acid	93	2	5

Triolein labeled with C^{13} (in the fatty acid moity) or labeled oleic acid was fed to a human subject with chyluria. The chemical composition and C^{13} content was determined on the lipids excreted in the urine. Taken from Blomstrand and Ahrens: J. Biol. Chem., *233*:321, 1958.

in Table 41. No diglycerides and insignificant amounts of monoglycerides were found. The lymphatic vessel which joins the left renal pelvis was almost certainly an intestinal lymphatic and not the thoracic duct. Hence lymph originated directly from the small intestine and was not diluted by lymph from the lower extremities or other parts of the G.I. tract. In the oleic acid experiment 90 to 96 per cent of the labeled oleic acid appeared in the triglyceride and cholesterol ester fraction, 3 to 9 per cent in the phospholipid fraction, and 1 to 7 per cent as free fatty acids. The specific activity of the label in the lymph was about 90 per cent that in the material fed, indicating that most of the glyceride in the lymph was derived from the lipid fed. The phospholipids, however, contained only 20 per cent of the label, indicating that 80 per cent of these fatty acids were not derived from fatty acids in the lumen of the intestine. The workers concluded that the phospholipids appearing in the lymph cannot be the precursors of lymph triglycerides. Low specific activity of lymph fatty acids suggests that nonesterified fatty acids of the lymph must have been derived from other sources than newly absorbed fatty acids.

The chemical composition of the particles leaving the epithelial cells is not known. When they reach the lacteals they consist of particles of 1 μ in diameter, predominantly triglyceride, some phospholipid, and some free fatty acid. The particle is surrounded by a thin layer of protein which apparently is synthesized by the intestinal mucosa. Bragdon[47] found that after injection of labeled amino acids the protein in the thoracic duct chylomicrons became labeled faster than those in the peri

pheral blood. There are a variety of chylomicrons with different compositions in the thoracic duct. Hillyard et al.[88] have recently studied the composition of different particles under different conditions.

The ligation of the thoracic duct does not reduce lipid absorption.[3] This is undoubtedly the result of the rapid establishment of a collateral circulation[57, 107a] (see Chapter 1). Clarke, Ivy, and Goodman[57] found that after ligation of the left thoracic duct a large chyle-filled collateral channel developed which had not been present at the time of the original operation. Even tearing the intestinal lymphatics, as a method of removal, leads to extensive regeneration.

While the long-chain fatty acids are converted to triglycerides and absorbed through the lymphatics, the short-chain fatty acids are poorly esterified and appear mainly in the blood capillaries. Considerably less than one half of the decanoic acid appears in the lymph,[29, 22] while the remaining fraction appears in the portal vein.[104, 42] Tributyrin is absorbed exclusively by the portal route.[91] Similar data have been obtained in human subjects. Fernandes, Van de Kamer, and Weijers[72] found that while higher fatty acids appeared in the lymph very little decanoic and no octanoic appeared. The patient with chyluria studied by Blomstrand, Thorn, and Ahrens[27] did not excrete octanoic acid or its glycerides after ingestion of 20 gm. of a triglyceride of predominately octanoic acid. On the other hand, this patient absorbed oleic and palmitic acids predominately by the lymphatic route.

The rate of absorption of short-chain fatty acids is extremely rapid. Deuel, Hallman, and Reifman[68] have compared the rate of absorption of fatty acids of different chain length and concluded that butyric, caproic, and caprylic acids are absorbed more rapidly than acids containing an odd number of carbons. Smyth and his collaborators[9, 135] have published data on short-chain fatty acid absorption showing movement of the fatty acid across isolated rat intestine against a concentration gradient. They suggest that fatty acids are actively transported by the intestine. Hogben[90] has pointed out that these authors have not seriously considered the possibility that the pH difference across the gut wall might influence the fatty acid gradient. As discussed in Chapter 3, weak acids usually pass across cell membranes much more rapidly in their un-ionized form and consequently the rate of movement and the equilibrium will be influenced by the pH difference across the membrane. It may be calculated that by this mechanism a difference of 0.3 pH units (a concentration gradient for H ions of about 2) could give a concentration gradient of about 2 for a weak acid. Rat jejunum is more acid on the mucosal side when incubated *in vitro*, which is the condition that would favor the transfer of a fatty acid to the serosal side by the mechanism described. Although this mechanism may not be responsible for the

observed findings, it must be clearly excluded before classifying the absorption of short-chain fatty acids as active transport.

Mechanism of Triglyceride Synthesis from Fatty Acids

Two general mechanisms of glyceride synthesis might be considered, one involving the hydrolytic enzyme, lipase, and another involving more complex reactions, such as those in liver.[101] Although synthesis of ester bonds can occur by the action of lipase,[11] under physiologic conditions there is never a net synthesis of ester bonds; instead, there is a net hydrolysis. The equilibrium depends upon the chemical composition of the substrates and products but in the case of glycerol and long-chain fatty acids the equilibrium leans heavily in the direction of hydrolysis. The concentration of fatty acids and glycerol or glycerides is never high enough to produce net synthesis in the physiological situation. Bergström et al.[16] have studied a compound (2,2-dimethylstearic acid) which is not a substrate of lipase but is readily absorbed and converted into triglyceride by the intestinal mucosa. In this case some mechanism other than lipase must have been operative.

As mentioned previously, the glycerol liberated by hydrolysis of triglycerides does not contribute to the resynthesis of triglyceride within the epithelial cells. Studies with glycerol labeled with deuterium,[70, 71, 19, 52] with C^{14},[81, 82, 113] or glycerol-labeled trilinolein[125] showed that free glycerol fed with fatty acids or hydrolyzed from triglycerides does not appear in the triglycerides of the chyle. Attempts to demonstrate a glycerol kinase by direct assay have failed[50, 148] although small amounts of glycerol are apparently metabolized.[128a] Buell and Reiser[51] studied homogenates of swine intestinal mucosa incubated with palmitic acid and C^{14}-labeled fructose diphosphate and found radioactivity in the glycerides. Unlabeled dihydroxyacetone phosphate or L-α-glycerophosphate reduced incorpora-

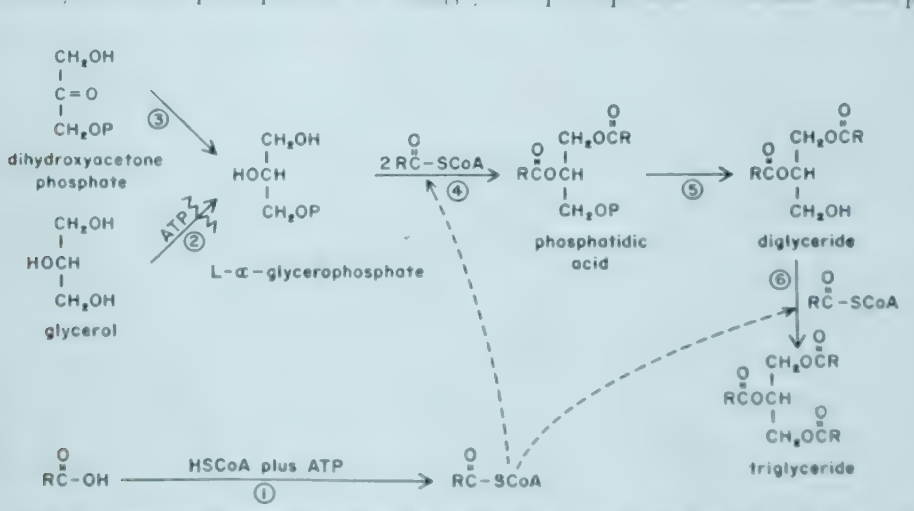

Figure 81. Triglyceride synthesis in intestinal mucosa. Step 2 occurs in liver but not in intestinal epithelium. (Kennedy, Ann. Rev. Biochem., *26*, 1957.)

Table 42. Fatty Acid Absorption by Sacs of Hamster Intestine

Location	Sac. No.	Mucosal solution		Gut wall		Serosal solution	
		Total activity	Per cent glyceride	Total activity	Per cent glyceride	Total activity	Per cent glyceride
Upper jejunum	(1)	51,000	18	11,000	73	3,000	90
	(2)	57,000	16	11,000	84	1,600	94
	(3)	59,000	22	9,300	83	840	—
	(4)	62,000	6	6,400	80	510	—
Low ileum	(5)	66,000	2	3,500	74	280	—

Sacs containing 0.5 ml. of bicarbonate-saline incubated in 4 ml. of a similar solution containing C^{14}-palmitic acid-albumin complex (70,500 cpm.). Modified from Johnston: Proc. Soc. Exper. Biol. & Med., *100*:669, 1959.

tion whereas glycolysis did not. They concluded that there is no glycerokinase and that glycolysis is the source of glycerol, probably by way of dihydroxyacetone (step 2 in Figure 81).

Johnston[92] has recently obtained data on fatty acid absorption with *in vitro* preparations of hamster intestine. Everted sacs of hamster intestine were incubated in a mucosal solution containing C^{14}-palmitic acid-albumin complex and triglyceride was found on the serosal side. As shown in Table 42, about 90 per cent of the radioactivity on the serosal side was glyceride, the remaining 10 per cent fatty acid. The upper portion of the intestine was most active in this regard. In a further study[94, 95] the glycerides were carefully fractionated on silicic acid columns and the glyceride fraction contained mainly triglyceride, some diglyceride and no monoglyceride (Figure 82). The lack of monoglyceride is in agreement with the studies of Blomstrand and Ahrens[23a] and is consistent with the pathway of triglyceride synthesis in liver proposed by Kennedy[101] (Figure 81).

The hypothesis that phospholipid is an intermediate in fat absorption has had a long and checkered history. The first to provide definite experimental support for this view were Bloor[32, 33] and Sinclair.[132] Later it was found that phospholipids increase in the chyle during fat absorption,[140, 48, 35] and, although the newly synthesized phospholipids contain dietary fatty acids, the fraction of ingested fatty acids in phospholipids is less than 5 per cent.[29, 36, 38] Experiments with P^{32} showed increased turnover of phospholipids during fat absorption[129] but it was thought[149] insufficient to account for all the lipid passing through phospholipid as an intermediate.

Apparently this failure to find convincing evidence for participation of phospholipid in fat absorption was due to the fact that only a small fraction, the phosphatidic acids, is directly involved in triglyceride synthesis. Furthermore, the concentration of this important intermediate

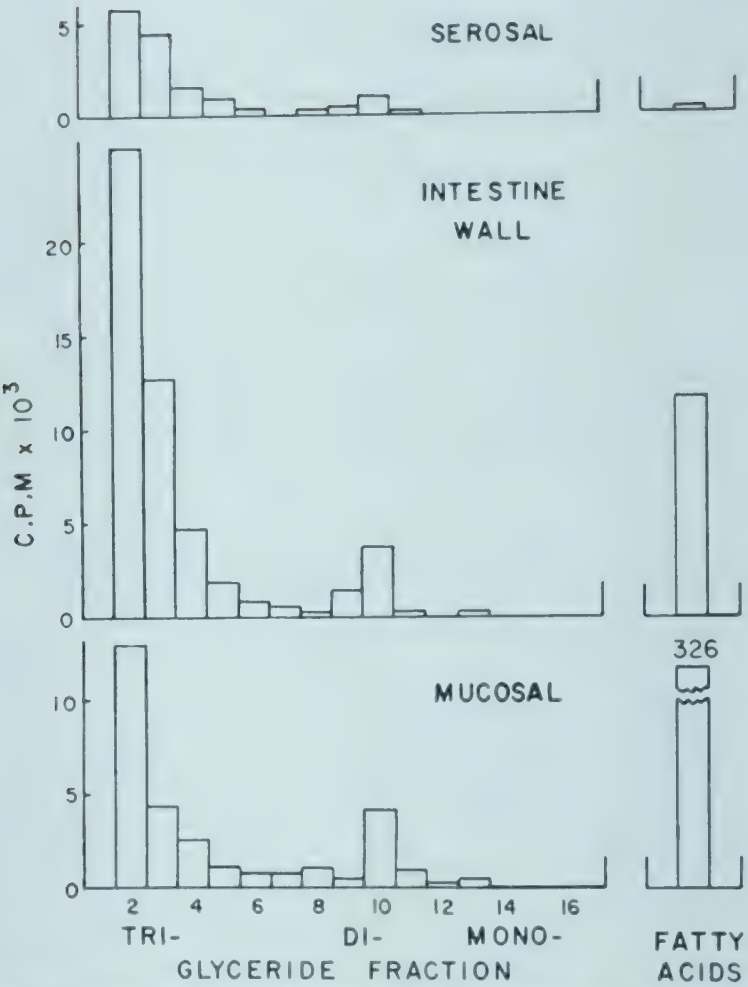

Figure 82. Glycerides formed during fatty acid absorption. C^{14}-palmitic acid was placed on the mucosal side of hamster intestine *in vitro*. Following incubation the glycerides were separated on silicic acid columns. (Johnston: J. Biol. Chem. *234*, 1959.)

is very low indeed. Johnston and Bearden have found that the phosphatidic acid fraction of the the phospholipids is the most active during fat absorption.[97, 98] Following incubation of intestinal segments in either C^{14}-palmitate or $NaH_2P^{32}O_4$, the phospholipid fraction containing the major activity was phosphatidic acid. The incorporation of P^{32}-phosphate into phosphatidic acid in the gut wall was increased two- to three-fold when fatty acids were included in the incubation mixture. These data provide convincing evidence that phosphatidic acids are indeed intermediates in triglyceride synthesis in the intestine (similar to the pathway in the liver[101]).

Dawson and Isselbacher[60-63, 91a] have obtained important evidence for the participation of coenzyme A (CoA) and adenosine triphosphate (ATP) in the activation of fatty acids prior to their esterification as triglycerides. Homogenates of rat or human intestinal mucosa, freed of nuclei and cell debris by centrifugation, were incubated with C^{14}-palmi-

tate for 30 minutes followed by isolation of glyceride fractions. Virtually no activity was found without the addition of CoA, ATP, and magnesium ions. Tween 80 and fluoride helped to inhibit the lipase which tended to hydrolyze the newly synthesized triglyceride. In both the rat and human the upper small intestine was most active in the incorporation of fatty acid into glyceride (Figure 81). In this system octanoate was 1/20 as active as stearate in giving rise to glycerides.[60]

The activation of fatty acids (step 1, Figure 81) has recently been studied as a separate step by Senior and Isselbacher.[130a] They observed that the microsomal fraction was most active in this reaction. This observation is consistent with the view that fatty acids can be converted to triglycerides within the endoplasmic reticulum of the cell.

Further evidence that the intestinal synthesis of triglyceride is similar to that in liver was the demonstration of a phosphatase which converts phosphatidic acid to diglyceride and inorganic phosphate. This conversion (at pH 6.2) by intestinal mitochondria and microsomes has been shown by Johnston.[96] Direct evidence for the participation of diglyceride in the sequence of reactions was the demonstration that diglyceride plus palmityl-CoA became triglyceride.[96]

Conversion of Monoglyceride to Triglyceride

Although appreciable quantities of monoglyceride enter the epithelial cell [125, 25, 134] and are converted to triglyceride, the biochemical pathway for conversion is not yet established. Clark and Hübscher[56, 56a] demonstrated that the addition of monoglyceride to a homogenate of intestinal mucosa fortified with cofactors and palmitic acid-C^{14} greatly stimulated the incorporation of palmitic acid to triglyceride. Tween 20, which strongly inhibits phosphatidic acid phosphatase, did not inhibit the monoglyceride pathway. The findings suggest that the monoglyceride pathway is distinct from that involved in conversion of fatty acids to triglyceride. Clark and Hübscher[56, 56a] propose that monoglycerides react directly with acyl-CoA to produce triglyceride without any phosphorylated intermediates.

An alternative hypothesis for the synthesis of triglyceride from monoglyceride has been suggested by Johnston[96] to explain his observations that ATP stimulates the incorporation of C^{14}-labeled palmitic acid-CoA into triglyceride in the presence of a homogenate of intestinal mucosa. He has suggested the possibility of phosphorylation of monoglyceride to lysophosphatidic acid, followed by acylation to form phosphatidic acid. The crucial experiment for this hypothesis, the isolation of lysophosphatidic acid from the system, has not been carried out. The data available at present are too fragmentary to decide between the two possible pathways of monoglyceride metabolism.

Other Enzymes of Lipid Metabolism in the Intestine

Long-chain alcohols, corresponding to natural fatty acids, are absorbed from the intestine and some of the alcohol is oxidized to the acid during transit through the epithelial cells. As early as 1891 Munk and Rosenstein[116] fed cetylpalmitate and found triglyceride in the lymph. Blomstrand and Rumpf[26] have reviewed the literature on cetyl alcohol (the alcohol corresponding to palmitic acid) and report work of their own indicating that there is an active oxidizing system in the intestinal mucosa, converting alcohol to acid. There is presumptive evidence for an aldehyde intermediate.

A most unusual enzyme for animal tissues is that which splits the ether link in chimyl alcohol (a glycerol ether with cetyl alcohol). When C^{14}-labeled chimyl alcohol was fed to rats[12] or to a human subject,[24] some was absorbed without hydrolysis and some was hydrolyzed and converted to palmitic acid. The conclusion was drawn that 50 per cent of the absorbed chimyl alcohol took the following pathway: (1) hydrolysis of the ether link to cetyl alcohol, (2) oxidation of cetyl alcohol to palmitic acid and (3) esterification of palmitic acid to produce tripalmitin.

PARTICULATE ABSORPTION

The possibility of particulate absorption has interested physiologists for 60 years. In 1900 Henriques and Hausen[85] fed an emulsion containing equal parts of lard and paraffin. The intestine rejected the paraffin completely and absorbed only the lard. This observation was confirmed by some[31, 108, 112] and denied by others.[54, 55, 109, 139] More recently Frazer was able to demonstrate absorption of paraffin if the particle size was reduced to 0.5 μ[76, 78] and claimed that the negative results of others were due to inadequate emulsification.

Recently, attempts have been made to demonstrate absorption of other hand there is evidence for the uptake of latex spheres,[128] insoluble size or form in which methylemethacrylate could be absorbed. On the other hand there is evidence for the uptake of latex spheres,[28] insoluble dye particles[8] and labeled resin particles.[118] The data of the last two studies are convincing, although it is difficult to assess their quantitative aspects.

MORPHOLOGICAL CHANGES ASSOCIATED WITH FAT ABSORPTION

Profound morphological changes can easily be observed in the epithelial cells of the small intestine during lipid absorption. As early

as 1842[81, 82] it was known that the epithelial cells become crowded with small and large particles of lipid, easily identified with a variety of fat-soluble stains. The smaller droplets appear in the apical or mid-portions of the cell and the larger ones tend to appear a little lower, just above the nucleus. These observations are completely consistent and have been repeated by many subsequent workers.[102, 5, 6, 87, 88, 117] As somewhat similar droplets are present in the intestinal lumen during fat absorption, it was perfectly reasonable for the early workers to assume that the cell had taken up the particles in the lumen by imbibition or phagocytosis.

The view of particulate absorption of fat was seriously challenged by the early biochemical experiments of Pflüger,[120-123] Bloor, [31-33] and others, which showed, among other things, that fatty acids or soaps fed to animals were transformed to neutral fat appearing in the lymphatics. These data seemed to indicate that water-soluble soaps diffused into the cells and were converted to triglycerides. How these triglycerides escaped from the cell did not seem to concern anyone.

Frazer[73-75] resurrected the particulate absorption hypothesis which again became fashionable for a number of years. During the past ten years biochemical studies once again have emphasized the extensive hydrolysis prior to absorption and the resynthesis of triglyceride within the mucosa. When the particulate hypothesis was at its lowest ebb, Palay and Karlin[117] published convincing electronmicrographs suggesting particulate absorption by pinocytosis.

Although a variety of workers such as Baker[5, 6] and Hewitt[87] have presented morphological evidence of particulate absorption, the elegant study of Palay and Karlin seemed to overshadow them. The investigation of the last two workers will be considered in some detail as it is the most complete study of its kind. In confirmation of earlier observations by Baker and Hewitt these investigators demonstrated that streaks of fat appear in the striate border during absorption. About 20 minutes after feeding 1.5 ml. of corn oil to a rat, the osmium-fixed tissues show that small droplets of fat (about 50mμ in diameter) are lodged in the spaces between the microvilli. Accumulations of these droplets presumably account for the streaks of fat seen under the light microscope. At this stage the pinocytotic vesicles (which apparently occur spontaneously in the absence of fat) are sometimes found to be filled with a droplet of lipid. In the apical portion of the cell, just below the terminal web, are seen many fat droplets 110 to 240 mμ in diameter, each enclosed in a thin envelope. The occasional observation of tubular extensions to these round, lipid-containing structures suggests that the droplets lie in a labyrinthine membranous system (endoplasmic reticulum). This is confirmed by the discovery, in some cells, of droplets enclosed in membranes studded with fine granules, apparently identical with the ergastoplasm.

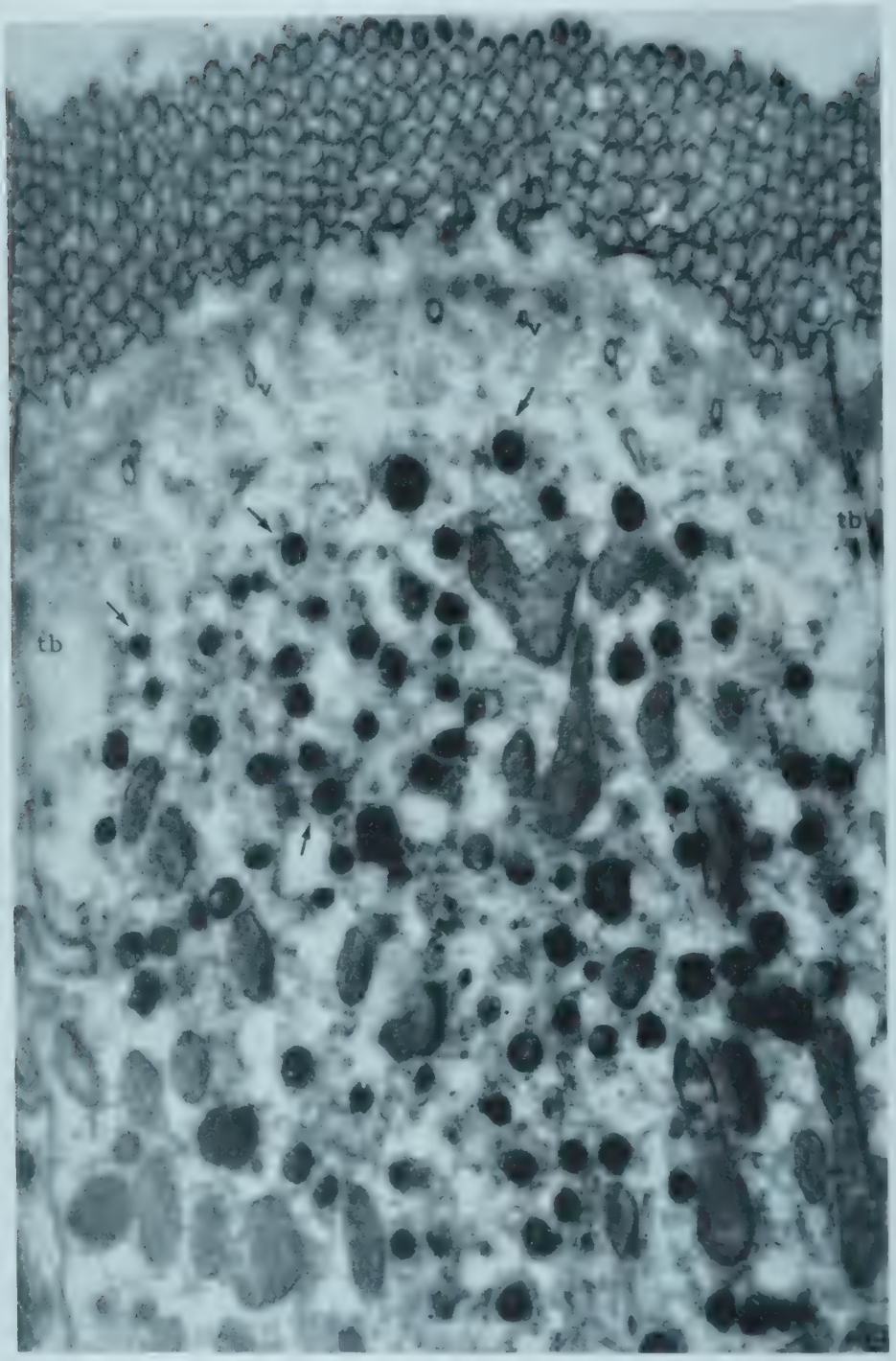

Figure 83. Striate border and apical cytoplasm of an intestinal epithelical cell from a rat that had been given 1.5 ml. of corn oil 75 minutes before fixation. In the terminal web and the subadjacent cytoplasm are small membrane-limited structures which are interpreted as pinocytotic vesicles (v) in the process of traversing the apical ectoplasm to join the endoplasmic reticulum. Deeper in the cytoplasm and filling most of the picture are individual fat droplets enclosed within a membranous envelope. x 28,000. (Palay and Karlin, J. Biophys. & Biochem. Cytol., *5*, 1959.)

Extracellular fat droplets between the epithelial cells, especially below the level of the nucleus, were first described by Kischensky[103] and recently emphasized by Hewitt.[87] These observations seemed to indicate that fat is absorbed by passage between the cells rather than through them. The question was clarified by the work of Palay and Karlin[117] who showed that lipid did not pass between the cells in the apical region but passed through the apical cytoplasm of the cell and was then extruded at the level of the nucleus. As shown in Figure 84, virtually all of the

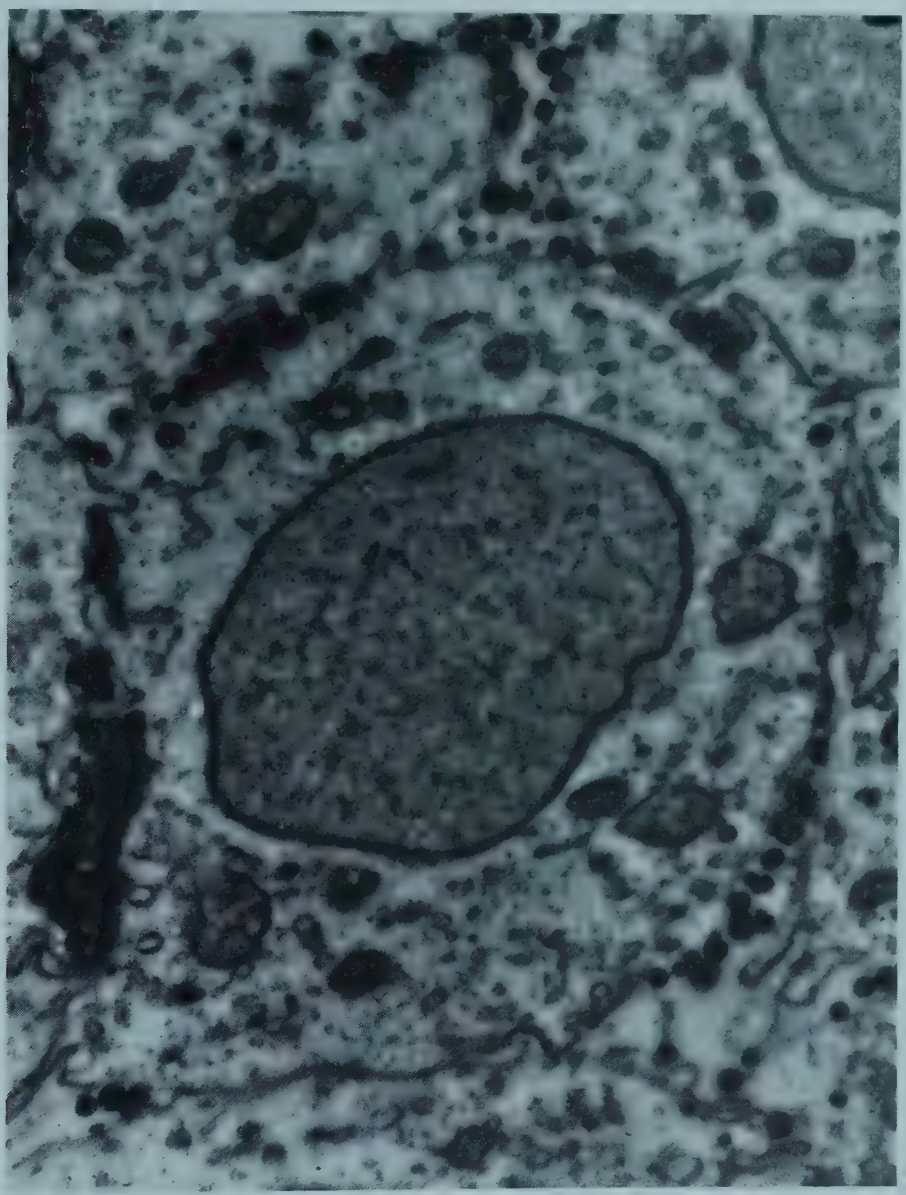

Figure 84. Extracellular position of lipid at the level of the nucleus 70 minutes after feeding corn oil. The section passes transversely across the cells at the nuclear level in a plane parallel with the free surface of the epithelium. Intracellular fat droplets are rare at this level while numerous droplets are present in the intercellular spaces. Note that the extracellular droplets are devoid of their membranous envelope. About x 13,000. (Palay and Karlin: J. Biophys. & Biochem. Cytol., *173*, 1959.)

lipid droplets are extracellular at the level of the lower half of the cells. The droplets cross the basement membrane and can be seen between the endothelial cells of the lymphatic capillaries.

THE PRESENT POSITION

Triglyceride in the lumen of the intestine is emulsified by peristalsis in the presence of bile salts to droplets fine enough to allow interaction with water-soluble lipase. Intraluminal hydrolysis results in the splitting of one third to one half of the molecules to fatty acids and glycerol, while the balance remains glyceride. The exact composition of the absorbed glyceride fraction is not known but it is presumed by many investigators that a major portion is monoglyceride.[89]

A mixture of fatty acids and glycerides enters the epithelial cell by either diffusion or pinocytosis or both. The fatty acids and lower glycerides are then converted to triglyceride, probably within the endoplasmic reticulum.[130a] The synthetic pathway from fatty acid proceeds by the addition of two fatty acids, through their Coenzyme A derivative, to endogenously produced glycerol phosphate. The resulting phosphatidic acid is dephosphorylated and a third acyl-Coenzyme A is added to the diglyceride to produce triglyceride. The pathway for conversion of monoglyceride to triglyceride is not yet definitely established.

The large particles of triglyceride (500 to 1000°A) within the endoplasmic reticulum are then extruded from the lateral margins of the cell by a process analogous to the pancreatic secretion of zymogen granules. The extracellular lipid droplets, free of endoplasmic reticulum, pass across the basement membrane and into the lymphatic vessels by passage between the endothelial cells.

There is now a large measure of general agreement on the process of fat digestion and absorption. One aspect of the problem not entirely resolved, however, is the question of the mechanism by which fat crosses the luminal membrane of the epithelial cell. Although there is evidence for lipid absorption by pinocytosis, the quantitative significance is difficult to assess at present. From what has been said in the previous chapter about lipid-soluble substances entering cells by solution in the lipid of the cell membrane, it is presumed that fatty acids and glycerides could diffuse into the cell if appreciable concentrations existed in a molecular form at the surface of the cell. Thus, the two opposing views of lipid absorption have still not been completely reconciled. To stimulate further experimentation in this area these two different interpretations will be discussed in more detail.

Pinocytosis Hypothesis

A good case may be made for the hypothesis that all lipid is adsorbed in the form of particles, about 500°A in diameter, by a mechanism of membrane invagination (Figure 85). According to this view fatty acids and glycerides arrange themselves in small particles, with the hydrophobic tails facing inwards and the hydrophilic ends outward. The composition of these particles would vary considerably in their proportion of fatty acid and glyceride. During transit through the cytoplasm of the cell, conversion of fatty acids to triglycerides probably occurs within the endoplasmic reticulum.

Although it is difficult to demonstrate lipid droplets within pinocytotic vesicles in the act of separation from the membrane, the demonstration of particulate absorption is unequivocal in the case of latex spheres[128] and insoluble dye particles.[8] In these two cases rarely is a piocytotic vesicle seen in the actual process of formation on the membrane although it must frequently occur. Therefore, inability to demonstrate the formation of the vesicle does not disprove this mechanism. Palay and Karlin[117] have made some interesting calculations which suggest that one cannot expect to see fat frequently in the process of entering the cell.

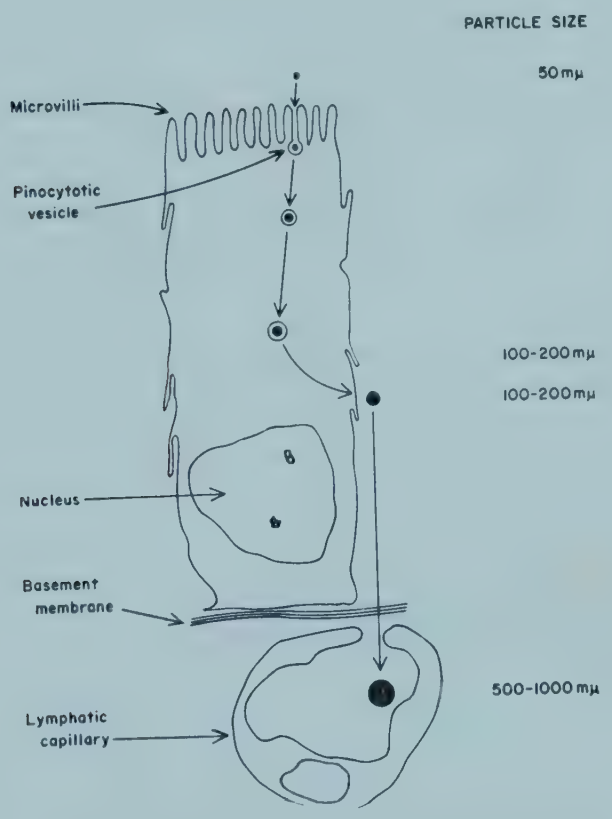

Figure 85. Pinocytosis hypothesis of lipid absorption.

Diffusion Hypothesis

An alternative view is that the major products of triglyceride hydrolysis, fatty acids and monoglycerides,[89] are absorbed by simple diffusion across the membrane of the epithelial cell by virtue of their solubility in the lipid portion of the cell membrane[90] (Figure 86). Chapter 3 emphasized the basic principle of cell permeability: a high degree of lipid solubility of a compound usually assures its ready entrance into cells (assuming the compound is in molecular form in the aqueous phase). One essential role of bile salts may be the emulsification of fatty acids and glycerides to molecular dimensions, a form which would permit diffusion into the cell.

In answer to the clear evidence of pinocytosis of latex and dye particles, one might argue that this phenomenon is entirely unrelated to lipid absorption. These foreign substances are probably absorbed in very tiny amounts by a process distinct from that for lipids.

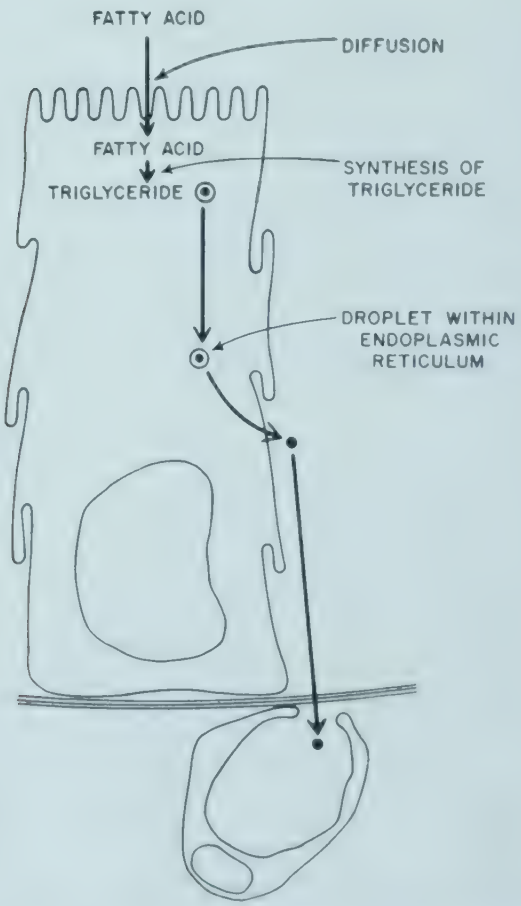

Figure 86. Diffusion hypothesis of lipid absorption.

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CHAPTER 8

Cholesterol, Bile Salts, and Phospholipids

CHOLESTEROL

The normal human diet usually contains approximately 0.5 gm. of cholesterol per day, although ten times this amount is sometimes ingested.[41] The limited capacity of the intestine to absorb cholesterol is in marked contrast to that for triglycerides. Ivy et al.[37] have found that an average of 2 gm. of dietary cholesterol can be handled daily, while it has been calculated that the maximum capacity for triglyceride absorption is 200 to 500 times this amount (see Chapter 1 for calculation). Man's efficiency in absorbing cholesterol has been studied by a number of workers.[37, 6, 33, 8, 60, 13] Hellman et al.,[33] for example, reported 52 to 86 per cent absorption of tracer doses of radioactive cholesterol and about 52 per cent for 1 gm. doses. In a patient with chyluria, Blomstrand and Ahrens[8] found that of an oral dose of 44 mg. only 20 per cent was absorbed in 15 hours.

On a cholesterol-free diet considerable quantities of cholesterol appear in the intestinal lymph, indicating absorption of endogenous cholesterol from the lumen. A variety of estimates of this recirculated cholesterol have been made.[19, 37, 53, 54, 8, 60] In man the excretion into the gut is

between 1 and 3 gm. daily. The dilution of radioactive cholesterol at various levels of the gut correlates well with the bile concentration and Borgström[14] concludes, therefore, that most of the endogenous cholesterol is derived from bile. Endogenous cholesterol comprises about one half the total cholesterol absorbed. Thus the recirculation (or enterohepatic circulation) is a prominent feature of cholesterol absorption. Experiments on mice[2, 16] show that it is also a feature of the absorption of certain cholesterol derivatives, the steroid hormones, testosterone, progesterone, and cortisone.

Lymphatic Route of Absorption

The absorption of cholesterol by the lymphatic route was established by Mueller[44, 45] and by Frölicher and Süllmann.[26] This has been confirmed and extended by recent quantitative studies which indicate that this sterol is absorbed exclusively into the intestinal lymphatics, both in animals[5, 18, 11] and in man.[32]

Delay in Cholesterol Absorption

Following a single oral dose of radioactive cholesterol the peak of specific activity in the blood is between two and three days in both humans[6] and rats.[29] Borgström, Lindhe, and Wlodawer[15] have shown that the slow release of cholesterol from the epithelial cells is responsible for the delay in its appearance in the blood. They fed tracer amounts

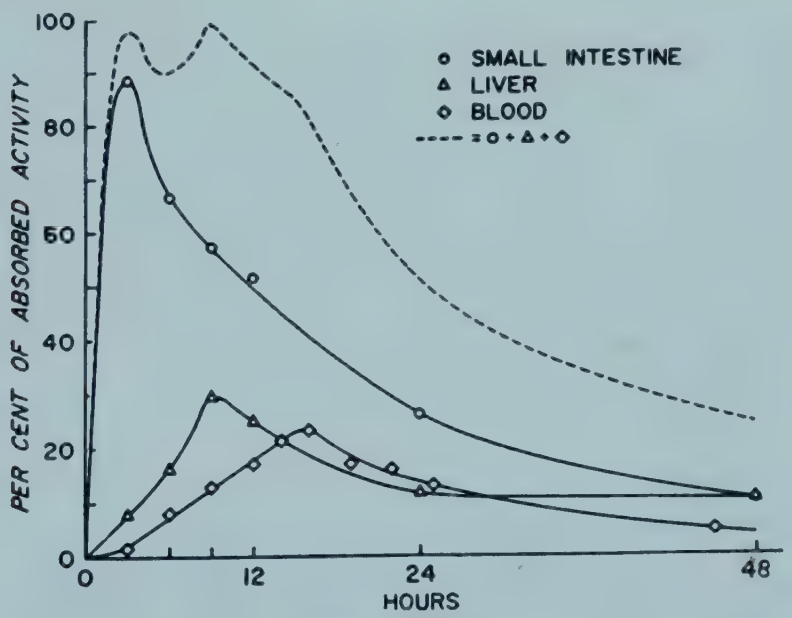

Figure 87. Percentage of absorbed cholesterol in different regions of the rat. Cholesterol-4-C^{14} (0.3 mg.) was incorporated into a well-balanced test meal and administered to each rat. Animals were killed at various time intervals after feeding, and radioactivity was determined. (Borgström et al.: Proc. Soc. Exper. Biol. & Med., *99*, 1958.)

of C^{14}-labeled cholesterol to rats and determined the radioactivity in the intestinal lumen, washed intestinal mucosa, liver and blood. Figure 87 shows that after three hours almost 90 per cent of the absorbed cholesterol was still present in the intestinal wall; after twelve hours 50 per cent still remained in the gut wall while 25 per cent was present in the liver and 20 per cent in the blood. This exceedingly long transit time for cholesterol across the columnar epithelial cell is another important point of difference between cholesterol and triglyceride absorption.

Role of Bile and Pancreatic Secretions

In 1916 Mueller[44] found that diversion of either bile or pancreatic juice from the digestive tract greatly reduced cholesterol absorption in the dog. There appears to be an absolute requirement for bile salts in the intestinal absorption of cholesterol.[57, 25] Figure 88 shows an experiment by Siperstein et al.[57] in which the diversion of bile completely inhibited cholesterol absorption into the lymph.

The experimental evidence demonstrating the importance of pancreatic secretion in cholesterol absorption is not as clear as that for bile salts. Although most workers have found that diversion of pancreatic secretion reduced absorption,[44, 36, 17] the opinion is not unanimous.[65] Hernandez, Chaikoff, and Kiyasu[36] found that loss of both pancreatic juice and bile led to complete cessation of cholesterol absorption. When pancreatic juice was fed with cholesterol no absorption was noted. Bile

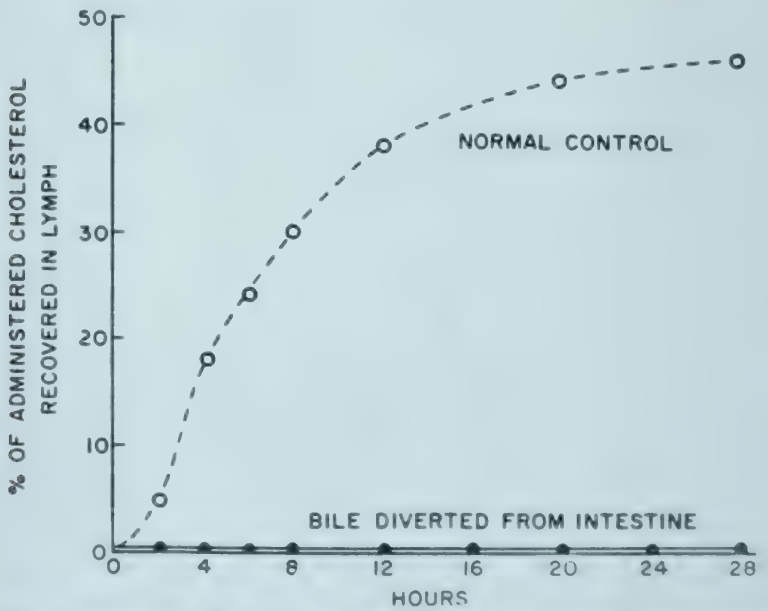

Figure 88. Importance of bile for cholesterol absorption. Cholesterol-C^{14} fed to two lymph fistula rats, one with normal bile duct and one in which the bile duct was cannulated and all bile diverted from the intestine. Radioactivity appearing in lymph was measured. (Modified from Siperstein et al.: J. Biol. Chem., *198*:111, 1952.)

alone produced some absorption but bile and pancreatic juice together gave best results.

Cholesterol esters present in the diet are probably largely hydrolyzed in the lumen prior to absorption. Schönheimer and Hummel[55] fed cholesterol oxalate and found considerable cholesterol in the liver but no trace of the oxalate, which suggested extensive hydrolysis prior to absorption. This and other evidence[36] suggests that pancreatic esterase causes extensive splitting of cholesterol esters in the lumen of the intestine.

EFFECT OF DIETARY LIPID ON CHOLESTEROL ABSORPTION

A number of earlier workers[21, 51] believed that no cholesterol was absorbed in the absence of triglyceride or fatty acid in the diet. There is now ample evidence that cholesterol alone is moderately absorbed in the rabbit[23, 49] and rat.[11, 48, 39, 68] Many dietary lipids stimulate cholesterol absorption and this effect has been the subject of considerable investigation. Kim and Ivy,[39] using balance methods in rats, found that only when the ratio of triglyceride to cholesterol was 24 or more was there significant stimulation of cholesterol absorption. On the other hand, they found fatty acids had a marked stimulatory effect and suggested that triglycerides stimulated through the fatty acids released by hydrolysis in the intestine. This effect of fatty acids has been confirmed and extended by Vahouny and Treadwell[68] who showed that oleic and linoleic acids stimulated cholesterol absorption in rats while stearic, palmitic, lauric, and butyric did not. The only triglyceride that stimulated absorption in this system was the one containing linoleic acid. If the stimulatory effect of fatty acids were due to the esterification of cholesterol in the lumen of the intestine, cholesterol esters should be absorbed more readily than free cholesterol. But this was not the case. Cholesterol oleate and cholesterol stearate were absorbed somewhat more slowly than free cholesterol.[55, 67, 47, 61, 48] A commonly held view is that fatty acids aid in the transport of cholesterol through the cell, or the exit from the cell, by providing an essential substrate for esterification of cholesterol.

Role of Esterification in Absorption

That esterification of cholesterol occurs during transit through the epithelial cell has been known since 1916.[44] After feeding free cholesterol, about two thirds of the cholesterol appearing in the chyle is in the esterified form. Pancreatic juice contains a very active enzyme which splits (or synthesizes) cholesterol esters, especially those with long-chain fatty acids. At equilibrium, approximately two thirds of the cholesterol is esterified

Table 43. Distribution of Cholesterol Esterase

TISSUE	CHOLESTEROL BUTYRATE	CHOLESTEROL OLEATE
Pancreas	242	75
Intestine	6	1.3
Liver	4	0
Kidney	0.5	0
Other organs	0	0

Activity measured as mg. cholesterol ester hydrolyzed per gm. tissue per hour in the presence of taurocholate. Taken from Swell, Boiter, Field, and Treadwell: Am. J. Physiol., *181*:193, 1955.

and a third free. In a study of the distribution of this enzyme in the rat, Swell et al.[62] found that, while the highest concentration was in the pancrease, considerable amounts were also present in the small intestine (Table 43). In a study of the substrate specificity of the esterase, Vahouny and Treadwell[67] found a compound, cholesterol trimethylacetate, which was not a substrate for the enzyme and was not absorbed. These and other observations have led many workers to believe that cholesterol esters are hydrolyzed in the lumen and the cholesterol must be re-esterified in the epithelial cells.

Evidence against the essential nature of the esterase reaction in absorption of cholesterol was provided by Daskalakis and Chaikoff.[22] These authors found that two sterols (dihydrocholesterol and ergosterol) which inhibited cholesterol absorption did not reduce the percentage of cholesterol appearing in the lymph in the esterified form. These compounds apparently compete with cholesterol for absorption at some step prior to the esterification reaction.[22, 66] Another study from Chaikoff's laboratory[36] has raised the possibility of simple adsorption of pancreatic esterase by intestinal epithelial cells, since pancreatectomy or diversion of the juice reduces the enzyme level in the intestinal mucosa. Borgström et al.[15] found, in following a tracer dose of C^{14}-cholesterol, that the sterol appearing in the thoracic duct during the first three hours was predominantly in the free form. The proportion of free to esterified in the intestinal mucosa was about four to one at 3 hours, one to one at 12 hours and one to three at 24 hours. They inferred that cholesterol enters the cell in free form and then is slowly esterified within the epithelial cell.

Mechanism of Cholesterol Absorption

Although there are not enough data available to support a comprehensive hypothesis of the mechanism of cholesterol absorption, a "working hypothesis" may be useful. The one given here is a composite of the views of many different authors. In our present state of knowledge it seems unnecessary to postulate any mechanism of entrance into the epithelial cell other than simple diffusion of cholesterol through solution in the lipid portion of the membrane. According to this hypothesis bile

salts are essential for emulsification and reduction of particle size to molecular dimensions, at which point diffusion into the membrane could occur. Pancreatic juice, which is less essential than bile, may aid in absorption by providing additional emulsifying agents from the hydrolysis products of triglycerides, such as fatty acids and lower glycerides. Fatty acid stimulation of cholesterol absorption might be explained in this manner. Movement through the epithelial cell might occur by some type of adsorption-desorption to compounds such as lipoproteins as suggested by Glover and Green,[28] which might account for the exceedingly slow movement. They suggest this lipoprotein step as the site of competition between plant sterols and cholesterol. Esterification within the cell is probably not the main control of absorption and may be only a fortuitous event. Epicholesterol is absorbed unesterified[35] and dihydrocholesterol is poorly absorbed,[29] though readily esterified.[63] As cholesterol is present in the chylomicra of intestinal chyle, this sterol may enter the triglyceride droplets in the cell and leave by the secretory route described for triglycerides. Perhaps fatty acids stimulate cholesterol absorption by providing an accessible vehicle to use in leaving the intestinal epithelium.

BILE SALTS

It has been known for many years that a fraction of the bile acids secreted into the lumen of the intestine through the bile was resorbed by the intestine, to be resecreted in the bile.[4] This cyclical process has been termed the enterohepatic circulation. When labeled taurocholic, glycocholic or glycodeoxycholic acid was fed to a bile fistula rat, 80 to 90 per cent of the bile acid was secreted in the bile within two hours.[50, 58] It was calculated by Bergström and Danielsson[3] that the bile acid pool of the rat circulates ten times daily. There is, of course, some loss of bile acids which must be replaced by liver synthesis but the recirculation process is quite efficient. A scheme of bile acid metabolism is given in Figure 89.

As a human normally absorbs 20 to 30 gm. of bile acids daily, the process of absorption is considerably more efficient than that for the parent sterol, cholesterol. Bile salts exist conjugated with either glycine or taurine and thus are more polar and more water soluble than many other sterols. Recently, Lack and Weiner[42] carried out an interesting study of bile salt absorption by isolated small intestine of the rat and guinea pig. Everted sacs of low ileum of both animals transported taurocholic acid across the gut wall against a concentration gradient of five- to fifteenfold. Sacs from the more proximal locations of the intestine did not transport bile salts (Figure 90). These workers have extended their studies to *in vivo* experiments on anesthetized guinea

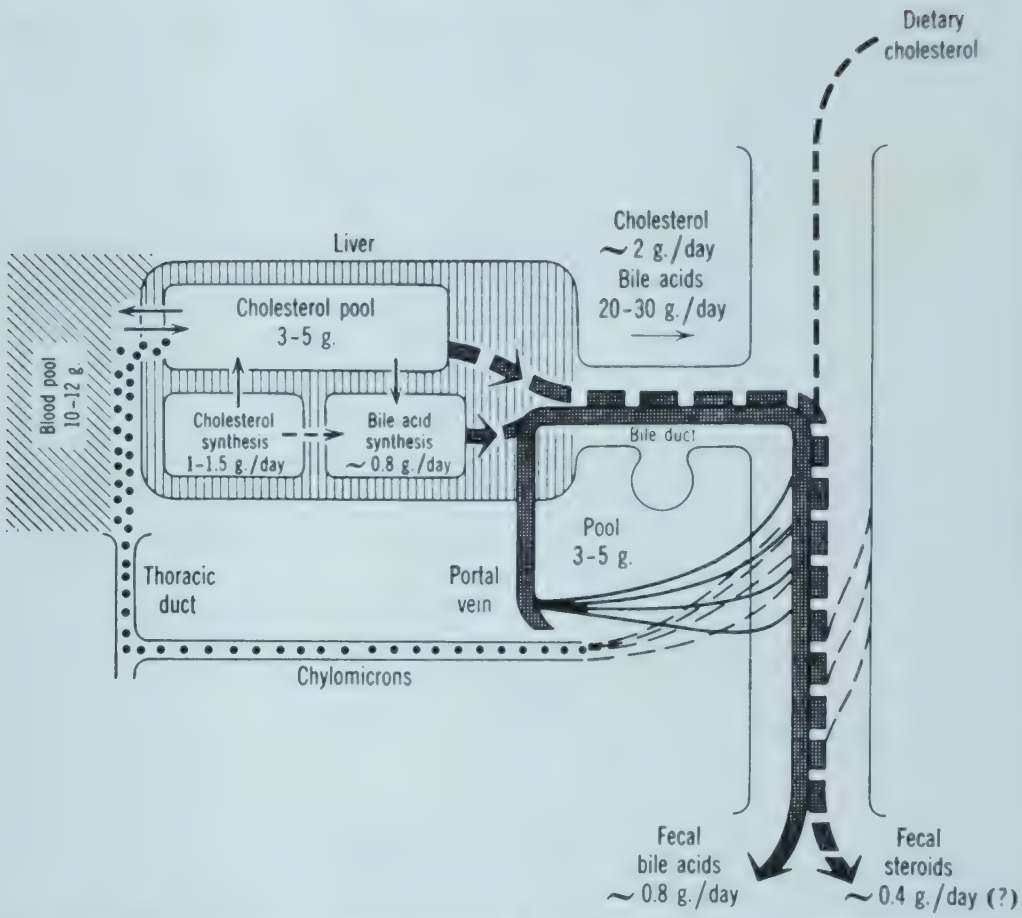

Figure 89. Summary of the turnover of cholesterol in man. (Bergström: In Ciba Foundation Symposium on the Biosynthesis of Terpenes and Sterols, Little, Brown & Co.)

pigs.[70] They placed bile salts in tied loops of either jejunum or ileum and measured the excretion into the bile by cannulating the bile duct. Taurocholate, glycocholate and cholate were all well absorbed from the ileum while virtually no absorption took place in the jejunum.

MISCELLANEOUS LIPIDS

The intestine possesses a remarkable degree of discrimination in its absorption of a variety of sterols of somewhat similar chemical structure. Vitamin A and cholesterol are absorbed to a moderate degree while certain plant sterols are not absorbed. Schönheimer and Hummel[53, 54] were the first to draw attention to this interesting phenomenon. Table 44 shows data taken from Sperry and Bergman[59] who obtained an indirect measure of sterol absorption by estimating the increase in liver sterol. Cholesterol feeding increased liver sterol while ostreasterol (derived from the oyster) produced only a small increase. Sitosterol gave no

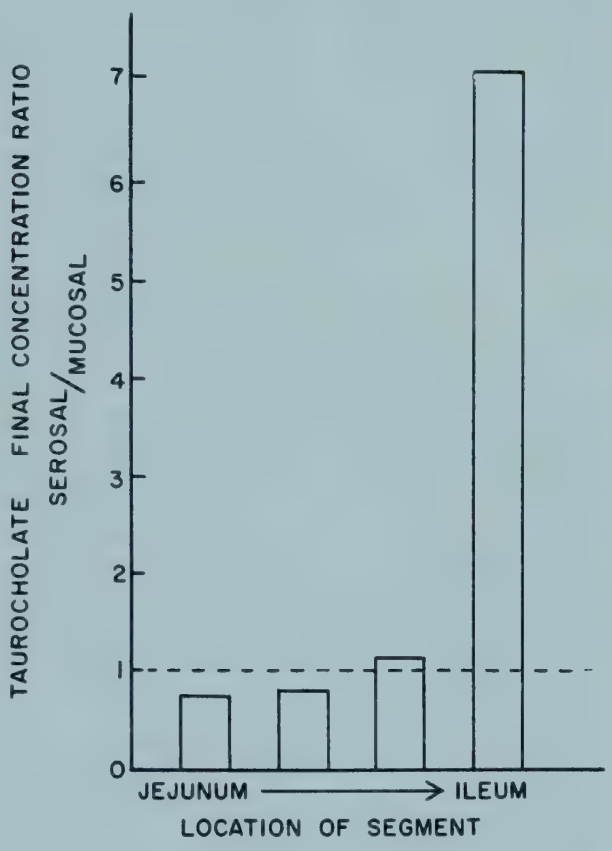

Figure 90. Location in the intestine of bile salts transport. Everted sacs of rat intestine were incubated with taurocholate-C^{14} on both sides of the intestinal wall. Final concentration ratio (serosal/mucosal) was determined. (Drawn from the data of Lack and Weiner: Am. J. Physiol., *200*:313, 1961.)

increase. The phytosterols (sitosterol and stigmasterol) not only are not absorbed but also block the absorption of cholesterol in animals[46] and in man.[8] This suggests that the inhibitors may compete for a common step in absorption.

It has been suggested by Ganguly et al.[27] that vitamin A and carotenoids are absorbed by attachment to specific lipoproteins of the intestinal mucosa, followed by transport across the cell, perhaps still associated with lipoprotein. Krinsky, Cornwall, and Oncley[40] have demonstrated that, following a meal, human blood contains different lipoproteins which adsorb vitamin A ester, vitamin A alcohol, and carotinoids, while chylomicra are essentially free of these lipid components. These authors emphasize the specific attachments of lipoproteins to these compounds.

Table 44. Specificity of Sterol Absorption

Sterol in diet	No. of animals	Sterol content of liver (%)
None (control)	8	0.61
Cholesterol	9	4.27
Ostreasterol	9	1.06
Sitosterol	10	0.40

Data taken from Sperry and Bergman: J. Biol. Chem., *119*:171, 1937.

Although most steroids are absorbed into the lymphatics, certain steroid hormones enter the circulation through the portal vein. In a man with a thoracic duct fistula, labeled hydrocortisone and testosterone, given orally, did not appear in the lymph although large amounts were soon recovered in the urine.[31] Presumably all of these compounds had passed into the portal vein.

PHOSPHOLIPIDS

The pancreas and the intestinal epithelium both produce a variety of enzymes capable of phospholipid hydrolysis. The nomenclature for these enzymes used in this chapter is taken from the excellent review of Kates.[38] Figure 91 indicates the linkage of the phospholipid molecule which is split by the different phosphatidases. It includes the recent observation that contrary to the previous belief phosphatidase A splits the fatty acid from the beta position of the glycerol.[64, 30] Phosphatidase B splits both fatty acid ester bonds, yielding glycerol phosphate esters.

Although the pancreas secretes both phosphatidase A[38] and phosphatidase B,[43] the former is probably more important *in vivo* in intestinal digestion as considerable free lysolecithin has been found in the duodenal contents.[12] Vogel and Zieve[69] have obtained pancreatic phosphatidase A from human duodenal contents and demonstrated both fatty acid and lysolecithin as products of lecithin hydrolysis. The enzyme that splits the diglyceride-phosphate link in a glycerolphosphatide (phosphatidase D) is present in some animal tissues[56, 38] and probably exists in the pancreas.

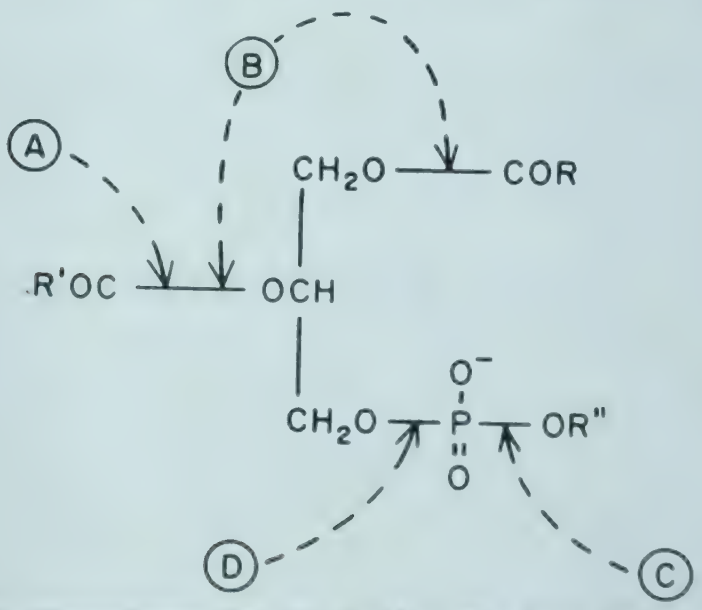

Figure 91. Nomenclature for phosphatides. The letter indicates the site of hydrolysis of phosphatidases with these letter suffixes. (Kates in Lipide Metabolism, John Wiley & Sons.)

Schmidt et al.[52] have found that phosphatidyl ethanolamine and phosphatidyl serine fractions of crude brain cephalin are hydrolyzed by a phospholipase in mitochondria of rat intestinal mucosa. Cephalin was hydrolyzed much faster than lecithin in this preparation and acetyl phospholipids were unaffected. Presumably this enzyme is not responsible for extracellular digestion as it occurs in the mitochondria within the cell. A particulate preparation of phosphatidase B has been studied by Epstein and Shapiro.[24]

A tentative scheme for the major pathway of hydrolysis of most phospholipids is given in Figure 92. Following the cleavage of the two fatty acid ester linkages a diesterase of the intestinal mucosa splits the glycerylphosphoryl compound to glycerol phosphate.[38] The final cleavage of the phosphate group is presumably carried out by the nonspecific phosphatase of the intestinal epithelium.

The most prominent feature of digestion and absorption of phospholipids is the extensive hydrolysis in the lumen of the intestine. When phospholipids are fed to rats the triglyceride concentration of the chyle increases while there is little or no increase in phospholipid.[1, 7, 10] Bloom et al.[10] found that following oral administration of phospholipid containing C^{14} fatty acids, 87 per cent of the absorbed radioactivity appeared in the lymph and only 20 per cent of this activity was in the phospholipids (Table 45). This indicated that a small fraction of absorbed phospholipid escaped hydrolysis and was absorbed intact. This confirms the previous study of Artom and Swanson,[1] who performed experiments with phospholipids labeled with P^{32}. When 11 gm. of lecithin was fed to a human subject with chyluria no increase in chyle phospholipid was noted.[9]

Virtually no phospholipid is present in the feces of rats that are fasting or on a fat-free diet. Ingestion of corn oil or oleic acid greatly

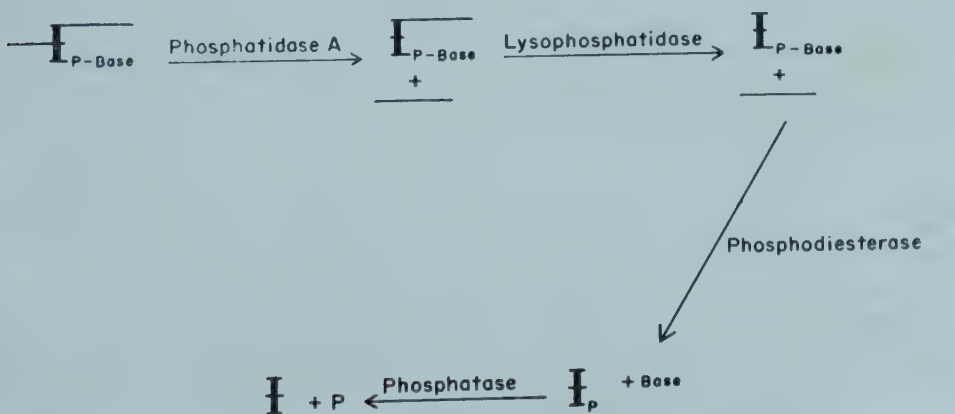

Figure 92. Probable pathway of hydrolysis of phospholipids in the intestine. Vertical heavy line indicates glycerol; horizontal line, fatty acid; *P*, phosphate; *B*, choline, ethanolamine (also possibly serine or inositol).

Table 45. Recovery of Phospholipids in Lymph

FED	ADMINISTERED P-LIPID-C^{14} (% ABSORBED)	ABSORBED C^{14} RECOVERED IN LYMPH FATTY ACIDS (%)	LYMPH FATTY ACIDS —C^{14} PRESENT AS PHOSPHOLIPIDS (%)*
C^{14} phospholipid 20 mg. plus 0.5 ml. corn oil	83	87	20

*Fatty acid-C^{14} in lymph phospholipides/total fatty acid-C^{14} of lymph X 100.
Taken from Bloom, Kiyasu, Reinhardt, and Chaikoff: Am. J. Physiol., *177*:84, 1954.

increases phospholipid excretion, oleic acid being most potent. Olive oil and oleic acid also increase the level of phospholipid in the lymph.[39,68] The significance of these interesting observations is poorly understood.

It is concluded that the major portion of phospholipids is completely hydrolyzed in the lumen of the intestine to fatty acids, glycerol, phosphate, and other compounds. A small fraction escapes hydrolysis and is absorbed intact into the epithelial cell, presumably leaving the cell in the triglyceride droplets which go to make up the chylomicra of the chyle.

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CHAPTER 9

Nucleic Acid Derivatives

Although there is now considerable information on the synthesis of nucleic acids there is much less data concerning their breakdown, especially in the gastrointestinal tract. Until very recently there had been few attempts to discover the chemical form of the nucleic acid derivatives absorbed from the small intestine.

HYDROLYSIS OF NUCLEIC ACIDS

Levene and Medigreceanu[11] in 1911 proposed the first clear classification of enzymes responsible for the hydrolysis of nucleic acids. They distinguished between nucleases, nucleotidases, and nucleosidases. Jones[7] was the first to demonstrate that nuclease cleaves internucleotide bonds without the liberation of inorganic phosphate or free bases. The products of nuclease action consist of a large series of polynucleotide fragments which cannot be further hydrolyzed by this enzyme. The small intestine possesses one or more phosphodiesterases which reduce the polynucleotides to individual nucleotides (see review by Khorana[9]).

Studies of London and Schittenhelm[12] in St. Petersburg and Levene and Medigreceanu[10, 11] at the Rockefeller Institute established the fact that rapid dephosphorylation of nucleotides occurred within the small intestine. An interesting experiment is one by the Rockefeller group[10]

who incubated guanylic acid with a sample of intestinal secretion sent by Pavlov from St. Petersburg. The solution of enzyme and guanylate soon became turbid and on standing crystals of pure guanosine formed. Associated with the formation of guanosine was the appearance of an equivalent amount of inorganic phosphate. All of the purine and pyrimidine nucleotides are rapidly hydrolyzed to the nucleoside in the small intestine. Whether there is a specific nucleotidase separate from the nonspecific alkaline phosphatase is not known.

Hydrolysis of nucleosides to free bases has been known for many years but not until 1947 was its mechanism elucidated. Kalckar[8] showed that instead of a hydrolysis the reaction was phosphorylysis. The reaction is:

$$\text{Nucleoside} + \text{phosphate} \rightleftharpoons \text{free base} + \text{pentose-1-phosphate.}$$

Friedkin and Roberts[3, 4] have studied such an enzyme in the small intestine that reacts with either thymidine or uridine. All available data are consistent with the view that nucleoside phosphorylase reactions are responsible for conversion of nucleosides to free bases in the intestine. For example, *in vitro* studies[22] have shown that during the formation of free base from uridine the ribose moiety disappears. As free ribose is not utilized by the tissue it is inferred that ribose derived from the hydrolysis of nucleoside is not in the free form but presumably exists as ribose-1-phosphate within the cell.

Pyrimidine Nucleotides

The hydrolysis of pyrimidine nucleotides by hamster intestine was recently studied by Wilson and Wilson,[22] using paper chromatography to separate the products of hydrolysis. When thymidine-5′-phosphate was placed on the mucosal side of everted sacs of hamster intestine there was a rapid hydrolysis to the nucleoside. There was an extremely slow hydrolysis of the nucleoside. In one experiment thymine was found only in small amounts.

Uridylic acid was hydrolyzed rapidly to the nucleoside which was slowly converted into uracil (Figure 93). The hydrolysis of cytidylic acid was somewhat more complicated than that of the other two pyrimidine

Table 46. Sequence of Steps During Hydrolysis of Nucleotides by the Gut

Pyrimidines:
1. Thymidylic acid → thymidine → thymine
2. Uridylic acid → uridine → uracil
3. Cytidylic acid → cytidine → uridine → uracil
Purines:
1. Adenylic acid → adenosine → inosine → hypoxanthine → xanthine → uric acid
2. Guanylic acid → guanosine → guanine → xanthine → uric acid

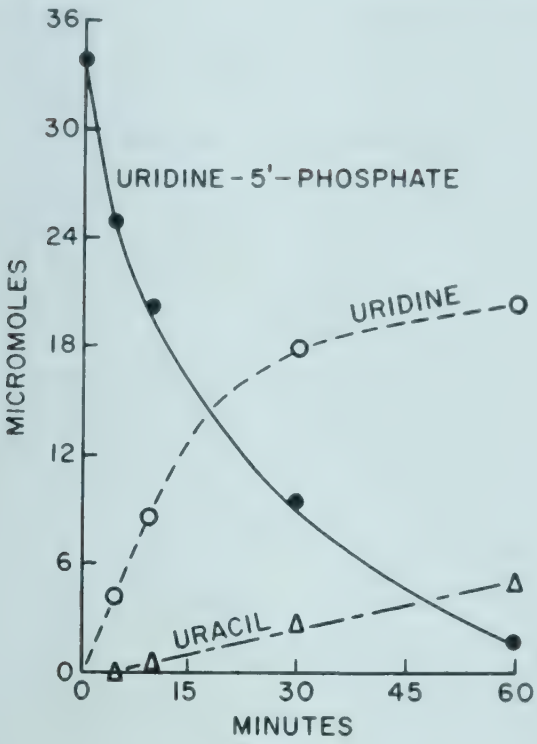

Figure 93. Uridine-5′-phosphate hydrolysis by rat intestine. An everted sac of rat jejunum was incubated for 1 hour at 37°C. in 3 ml. solution containing the nucleotide (10 mM.). (Wilson and Wilson: J. Biol. Chem., *233*, 1958.)

nucleotides as an additional deamination step is involved. Although the main product was the nucleoside, cytidine, some deamination of the cytidine occurred to produce uridine and some uridine was converted to uracil. The sequence of action is shown in Table 46. In agreement with this sequence, added cytosine did not increase the uracil.

Purine Nucleotides

Hydrolysis of purine nucleotides proved to be somewhat more involved than that for pyrimidines. Wilson and Wilson[21] found that incubation of hamster intestine with adenylic acid resulted in the production of as many as five different products, which could be separated by two chromatographic procedures. As with all of the nucleotides, the removal of the phosphate was extremely rapid (Figure 94). As large amounts of inosine and only small amounts of adenosine were produced, it was inferred that adenosine deaminase is an extremely potent enzyme in the intestine. Conway and Cooke[2] had previously shown that in the rabbit adenosine deaminase was most active in the appendix and jejunum, while the ileum, cecum, and colon were much less active. No inosinic acid was ever detected in the *in vitro* experiments so that deamination at the nucleotide level is unlikely. The conversion of inosine to hypoxanthine is probably carried out by a phosphorylase although the other product of the reaction, ribose-1-phosphate, was not isolated. Xanthine oxidase then converts hypoxanthine to xanthine and uric acid.

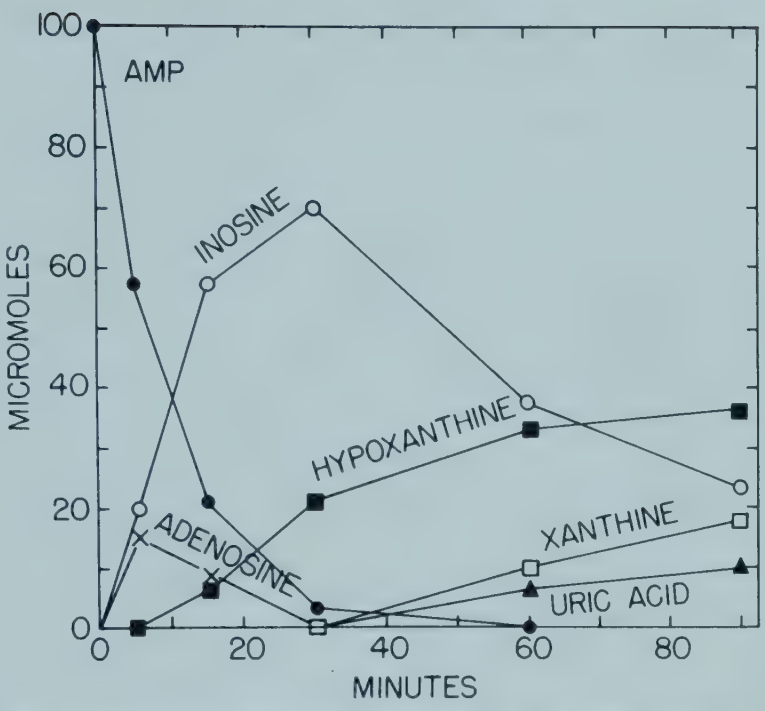

Figure 94. Adenosine-5′-phosphate hydrolysis by hamster intestine. An everted sac of hamster jejunum was incubated for 90 min. at 37°C. in 5 ml. of a solution containing the nucleotide (20mM.). (Wilson and Wilson: J. Biol. Chem., in press.)

The latter enzyme had previously been studied in the intestine by Morgan[14] and by Westerfeld and Richert.[20]

Incubation of intestinal segments in guanylic acid resulted in the formation of guanosine, xanthine, and uric acid (Figure 95). It is believed that guanosine is converted to guanine followed by conversion to xanthine. This view is supported by the observation that guanine is rapidly converted to xanthine while xanthosine (a possible intermediate) is not metabolized under these conditions.

Fate of Ribose and Deoxyribose

As mentioned above, ribose could never be recovered in appreciable amounts during conversion of uridine to uracil. As free ribose is not metabolized by the intestinal tissue, it was inferred[22] that the tissue had metabolized it in some other form. As a pyrimidine phosphorylase is known to be present in the tissue in large amounts, it is reasonable to suppose that ribose-1-phosphate was formed within the cell and metabolized. If a considerable fraction of nucleotides is reduced to the level of the free base prior to or during absorption, a corresponding amount of ribose will be made available to the intestinal epithelial cells in the form of the phosphorylated sugar. As ribose phosphate is readily metabolized and since cell membranes are virtually impermeable to organic phosphate, it is quite probable that most of the ribose is metabolized within

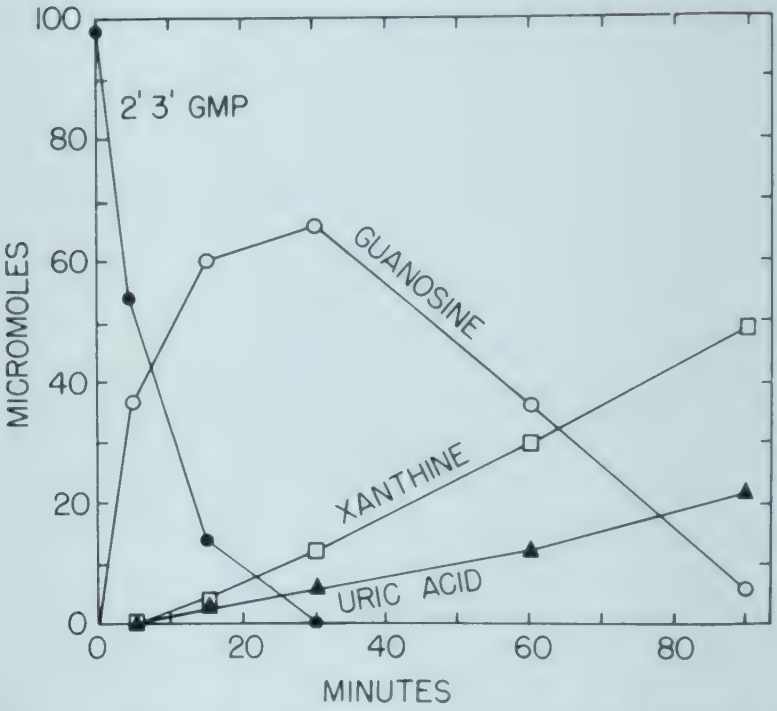

Figure 95. Guanosine-2′,3′-monophosphate hydrolysis by hamster intestine. An everted sac of hamster jejunum was incubated 90 min. at 37° C. in 5 ml. of a solution of the nucleotide (20mM.). (Wilson and Wilson: J. Biol. Chem., in press.)

the cell and not absorbed into the blood stream. If this view is correct it will provide a new and interesting pathway for the absorption of a dietary constituent.

ABSORPTION OF NUCLEIC ACID DERIVATIVES

Nucleic acids present in the diet are normally completely absorbed. A variety of early feeding experiments indicated that some intestinal absorption of the split products occurred before complete hydrolysis to free base. Thus, N^{15}-labeled nucleic acids[16] fed to animals were incorporated into the purines and pyrimidines of body nucleic acids while no incorporation was observed following ingestion of similarly labeled free bases, guanine,[15] hypoxanthine,[5] xanthine,[5] uracil,[15] thymine,[15] or cytosine.[1] Injection of N^{15} nucleosides[6] or nucleotides[6] into rats led to incorporation of isotope into body nucleic acids. The much greater incorporation of ingested nucleic acid than of free base indicates the absorption of some intermediate. It could not be determined directly from these studies, however, what fraction of ingested nucleic acids pass across the intestinal epithelium in the different chemical forms.

Although the previous data suggested that at least some of the fragments of nucleic acid digestion are absorbed in the form of nucleo-

tides and nucleosides, the intestine possesses considerable capacity for the absorption of the free bases. In 1910 Mendel and Myers[13] found that thymine, uracil, and cytosine were absorbed by the rabbit and man. Three gram doses of each of the three bases were ingested on separate occasions by one of the authors and the compounds were apparently completely absorbed.

When everted sacs of hamster intestine were incubated in the presence of a series of pyrimidine-5′-nucleotides, a mixture of nucleoside and free base appeared on the serosal side of the intestinal wall, but no nucleotide was detected.[22] Similar results were obtained with purine nucleotides.[21] The nucleoside probably moves across the wall by diffusion, as attempts to demonstrate active transport against a concentration gradient failed.

An active transport system in rat intestine for pyrimidine bases was discovered by Schanker and Tocco.[18] Thymine, uracil, 5-fluorouracil and 5-bromouracil were transported against concentration gradients by everted sacs of rat intestine (Table 47). The affinities of both uracil and thymine for the transport system are similar (K_t equals about 0.3 mM.). A study of the absorption of these compounds *in vivo* indicated that when the concentration in the lumen was above about 1 to 5 mM. considerable quantities were absorbed by diffusion, as the transport system had been saturated. Thymine and uracil apparently share the same transport system since uracil competitively inhibits thymine transport (Figure 96). The pyrimidine transport system is entirely separate from the sugar and amino acid pumps as shown by the fact that neither glucose nor L-histidine had any inhibitory effect on thymine transport (Table 48).

It would be interesting to know whether the purine bases share the same transport system. Hypoxanthine possesses considerable affinity for

Table 47. Active Transport of Pyrimidines

Pyrimidine	Conc. of pyrimidine (mM.)			Final conc. ratio serosal side / mucosal side
	Initial mucosal and serosal	Final mucosal	Final serosal	
Thymine	0.020	0.014	0.039	2.8
Uracil	0.020	0.012	0.056	4.7
5-Fluorouracil	0.020	0.011	0.049	4.5
5-Bromouracil	0.020	0.013	0.039	3.0

Sacs of everted jejunum were filled with 2 ml. of a solution containing a C^{14}-labeled compound and incubated for one hour in a beaker containing 15 ml. of the same solution. Data taken from Schanker and Tocco: J. Pharmacol. & Exper. Therap., *128*:115, 1960, and from Schanker and Jeffrey: Nature, London, *190*:727, 1961.

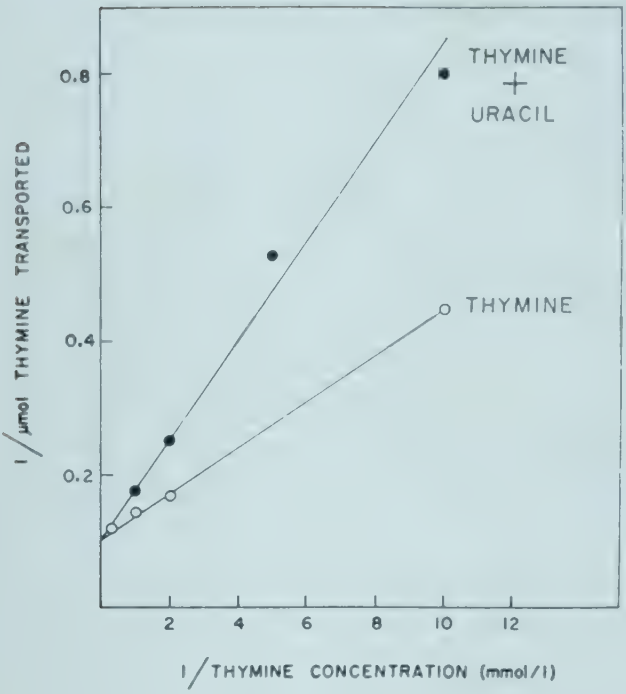

Figure 96. Competitive inhibition of thymidine transport by uracil. Thymidine-2-C^{14} (0.4 mM.) absorption was measured by a recirculation method in anesthetized rats. (Schanker: J. Pharmacol. & Exper. Therap., *128,* 1960.)

the system as it is a potent inhibitor of thymine transport (Table 48). On the other hand xanthine and uric acid are weaker inhibitors and adenine has little or no inhibitory effect.[19] Transport against a concentration gradient could not, however, be shown for hypoxanthine[16a] but its metabolism to xanthine and uric acid makes it unsuitable for transport studies. Some of the purines may enter the cell by the pyrimidine transport carrier and then may be metabolized.

Table 48. Affinity of Substances for the Pyrimidine Transport System (Inhibition of Thymine Transport)

Substance	Per cent inhibition of thymine transport at the following inhibitor concentrations	
	5 mM.	10 mM.
Uracil	99	100
Hypoxanthine	77	94
Cytosine	34	45
6-Azathymine	25	38
6-Azauracil	16	30
L-histidine	0	0
D-glucose	0	0

A 0.1 mM. solution of thymine-2-C^{14}, containing 5 or 10 mM. of a possible inhibitor was circulated through the small intestine of an anesthetized rat for one hour. The amount of thymine absorbed by the transport system was calculated. Data taken from Schanker and Tocco, J. Pharmacol. & Exper. Therap., *128*:115, 1960.

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CHAPTER 10

Proteins

In the preceding chapters considerable emphasis has been placed on the impermeability of the adult mammalian small intestine to large molecules. It has been stated that water-soluble, lipid-insoluble compounds with a molecular weight of a few hundred are absorbed poorly, if at all. In many lower forms of animals, however, (e.g., planaria), phagocytosis of large food particles is the primary means of ingestion. In the embryo and newborn mammal this more primitive mechanism persists. There is considerable variation in the length of time this phagocytotic or pinocytotic mechanism persists after birth, from a day or two (calf) to a few weeks (rat). Furthermore, the specificity of the ingestion process varies. Even in the adult mammal intact protein molecules are absorbed, but normally only in antigenic amounts. This absorption in the adult is insignificant from a nutritional point of view.

NEWBORN ANIMALS

From a teleological point of view the retention of the primitive function is exceedingly useful, if not essential, for certain newborn animals, since for these species intestinal absorption of antibodies of the colostrum is the sole means of transmission of passive immunity from mother to offspring.[5] This mechanism is poorly developed in animals that transfer antibodies through the placenta (e.g., human, rabbit, and

guinea pig), but for animals born without passive immunity against infection (e.g. ungulates, horse, and pig) the absorption of colostral antibodies is extensive (Table 49). The rat, mouse, dog, and cat comprise an intermediate group, obtaining some antibody transfer *in utero* and some after birth.

Table 49. Time of Transmission of Passive Immunity in Mammals

SPECIES	TRANSMISSION OF PASSIVE IMMUNITY	
	PRENATAL	POSTNATAL
Ox, goat, sheep	0	+++ (36 hr.)
Pig	0	+++ (36 hr.)
Horse	0	+++ (36 hr.)
Dog	+	+ (10 days)
Mouse	+	+ (16 days)
Rat	+	++ (20 days)
Guinea-pig	+++	0
Rabbit	+++	0
Man	+++	0

Taken from Brambell: Biol. Rev., *33*:488, 1958.

Apparently, the first demonstration of passive immunity from mother to young was by Ehrlich in 1892.[15] Subsequently, Culbertson clearly demonstrated passage of immunity from mother to offspring through colostrum in the rat.[10, 11] In the calf, the unexpected finding of proteinuria by Smith and Little[33] led to the discovery that newborn calves (up to three days old) absorb massive quantities of intact proteins of the colostrum and milk, the molecules of lowest molecular weights spilling out in the urine.[21]

Route of Absorption

In 1935 Alexander, Shirley, and Allen[1] demonstrated that the lymphatics were the route of absorption of egg white in adult dogs. Comline, Roberts, and Titchen[9] recently compared the thoracic duct with the portal vein as the route of absorption of colostrum protein in the calf. No absorption occurred by way of the portal vein. They found a 60 to 120 minute delay between introduction of colostrum into the duodenum and the appearance of colostral protein in the thoracic duct. As much as 1.2 gm. of colostrum proteins were obtained from the intestinal lymphatics in ten minutes. Similar observations were made with kids fed colostrum. May and Whaler[27] have found that botulinus toxin was absorbed through the lymphatics in the rabbit. They also demonstrated that diversion of lymph from the body by cannulation protected rats against toxicity.

Specificity

Considerable variation in specificity of protein absorption is seen in different animals. The calf apparently shows little discrimination between proteins.[14] It absorbs both albumin and globulin from colostrum at similar rates. In this regard the calf differs from the rat, which absorbs globulin 50 times as rapidly as it does albumin (Table 50). Halliday[16] has shown that the young rat absorbs antibodies derived from the same species most rapidily those from mouse and rabbit moderately well and those from the cow and fowl not at all. The pig has been shown[25] to absorb foreign proteins from the cow and chicken as well as polyvinylpyrrolidone, a nonprotein substance of a molecular weight of 20,000. On the other hand, there is an important quantitative difference between the absorption rates of different proteins. Payne[29, 30] has shown that the piglet absorbs gamma globulin from the colostrum of the cow, sheep, dog, and human at a very much slower rate than gamma globulins from pig colostrum. Intraperitoneal injection of all the above substances results in a prompt rise in blood gamma globulins in the piglet.

Table 50. Intestinal Absorption of Albumin and Globulin by Seven and Twenty-one Day Old Rats

Age of recipient rats (days)	Species of labeled serum given	Amount of fed albumin in the blood (%)	Amount of fed globulin in the blood (%)
7	Rat	0.12	5.1
	Rabbit	0.06	1.9
	Monkey	0.06	2.8
21	Rat	0.06	< 0.5
	Rabbit	0.04	< 0.5
	Monkey	0.02	< 0.5

Serum from three different animals was iodinated with I^{131} and fed to young rats. Radioactivity in the recipient's serum was determined three hours after feeding. Data taken from Bangham and Terry: Biochem. J., *66*:579, 1957.

Heterologous gamma globulins interfere with the intestinal absorption of homologous antibodies. This "interference" has been observed in the mouse[28] and the rat.[17] Rabbit, human, guinea pig, and bovine serums had a marked effect in reducing absorption of rat antibodies by the suckling rat. Mouse, hamster, and sheep serums do not interfere. Brambell et al.[4] have shown that this interfering effect is due solely to the globulins in the serums and not the albumins. The gamma globulin must be in considerable quantity in proportion to the immune globulin to be effective. It has been concluded[4] that globulins compete with one another for a common site of absorption in the intestinal mucosa. This suggests that the mechanism involves highly specific binding sites.

Duration of Protein Absorption

It has been known since 1924[33] that proteinuria in calves, and colostrum absorption, occurs only during the first two or three days after birth. Deutsch and Smith[14] have shown that the capacity for protein absorption in the calf is largely lost after the first 24 hours and that this period could not be prolonged by treatment with hormones or other measures. However, Halliday[17] showed that antibody absorption in the rat falls gradually from the first day to the eighteenth day and is completely lost at 21 days (Table 49). In mice, absorption of iodinated, homologous gamma globulin falls sharply between the fifteenth and seventeenth days. Cortisone has been found to shorten the period of protein absorption in the rat.[7, 16a] Payne[29] has found that the period after birth before protein absorption ceases can be extended by feeding the newborn pig a protein-free diet. Thus, animals fasted or fed glucose-water for three days will then absorb gamma globulin from colostrum. Once an animal's intestine has been exposed to protein it absorbs for 12 to 24 hours and then loses this capacity for the remainder of the animal's life.

Factors Protecting Proteins

Proteins may be partially protected from hydrolysis in the gut by trypsin inhibitor in the colostrum. The relative activity of the inhibitor varies with the species. Cow colostrum is ten times as active as human and sow colostrum is about 70 times as active as human.[23] The concentration of this inhibitor in the colostrum of the pig falls to almost zero in five days. This has led Laskowski et al.[23, 24] to suggest that antibodies are specifically protected against proteolytic activity during the first few days of life to facilitate absorption. In addition, Hill[18] has pointed out that in a number of animals free HCl is not produced for a few days after birth. This achlorhydria is an obvious advantage to a newborn animal absorbing antibodies.

Histological Changes During Protein Absorption

Smith in 1925[32] found that intestinal epithelial cells from newborn calves contained many vesicles or vacuoles which he believed contained protein. It is important to note that these peculiar vesicles disappeared at about the third day of life, which corresponds exactly to the cessation of protein absorption. These observations have recently been confirmed by Comline, Pomeroy, and Titchen,[8] who found the vesicles described by Smith also present in suckling pigs and kittens. In addition, animals which had not been suckled did not show the vesicles. Ingestion of colostrum apparently stimulates vesicle formation. Hill and Hardy[19] have made similar observations in sheep and goats.

An important advance in our understanding of these morphological changes has been made by Clark[7] who reinvestigated the problem in rats and mice with the electron microscope. He fed a variety of substances, some particulate, such as colloidal gold, saccharated iron oxide, India ink, and some purified proteins, such as bovine gamma globulin. The in

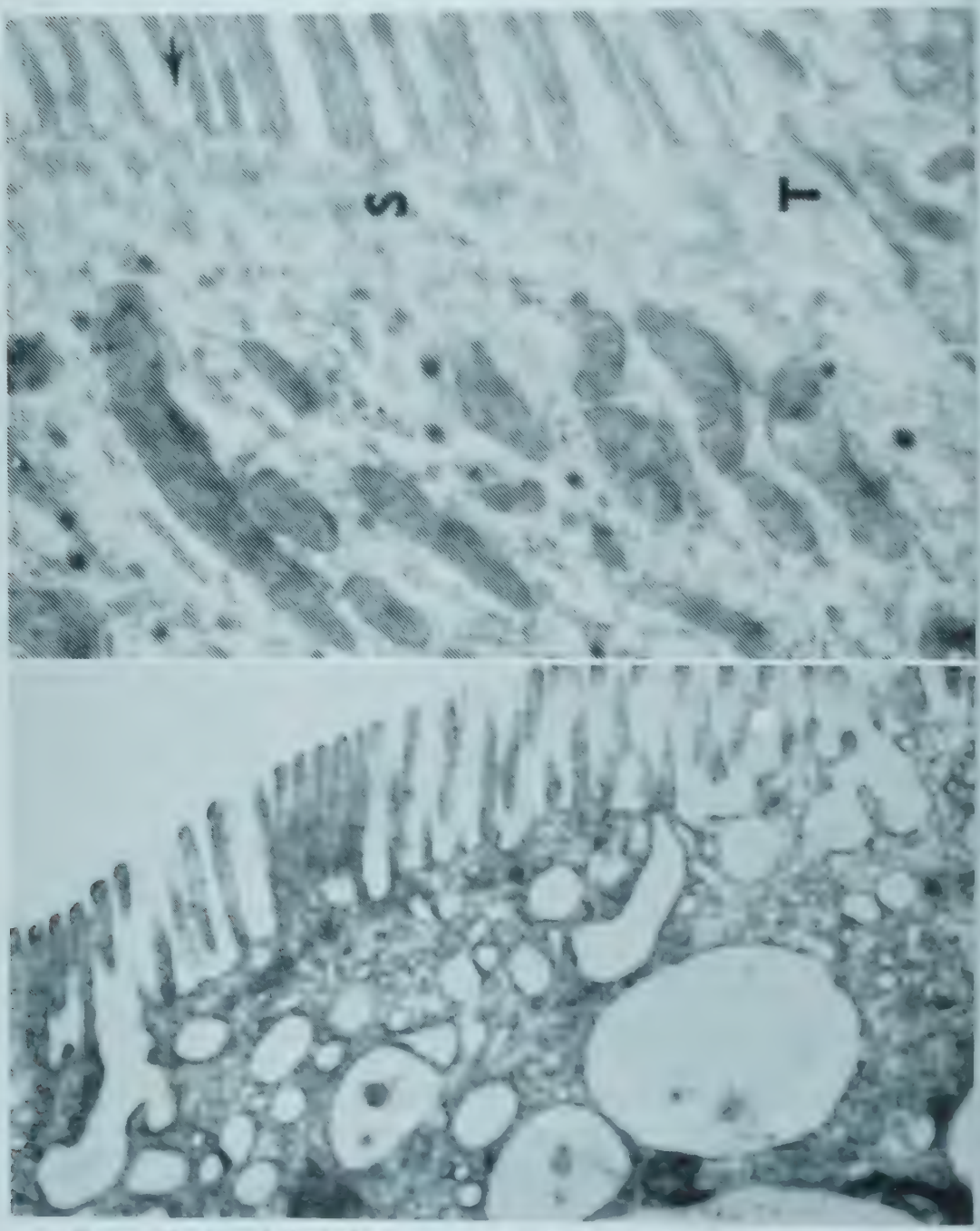

Figure 97. Morphological changes associated with protein absorption in the suckling rat. Apical cytoplasm of the intestinal epithelium following ingestion of protein by a 23 day old rat (above) and by a 8 day old rat (below). Approximately x 25,000. (Clark. J. Biophys. & Biochem. Cytol. 5. 1959.)

gestion of all of these substances stimulated vesicle formation. Pinocytosis was demonstrated by the appearance of particulate material in the small and large vesicles. Perhaps the best example of pinocytosis in animal cells is the electron micrograph of mouse gut ingesting colloidal gold (Figure 32). At about the twentieth day the rat no longer shows vesicles in the epithelial cytoplasm in response to the feeding of protein (Figure 97). It is concluded that protein absorption occurs by pinocytosis. In the ameba proteins are among the most active substances in stimulating pinocytosis.[20]

ADULT ANIMALS

Protein absorption in the adult animal has been known for many years (see reference 1 for review of work before 1936). In humans it is well known that allergic reactions can result from ingestion of the offending protein. Intestinal absorption of autogenous plasma proteins by dogs has been claimed by a number of workers[34, 31, 13] although not always confirmed.[26] Plasma absorption has not been reported in other animal species. Insulin, because of its simple biological assay, has been studied by a number of workers. Laskowski et al.[22] found that trypsin inhibitor allowed some absorption of insulin when 6 to 35 units were placed in isolated intestinal loops of 150 gm. rats. Danforth and Moore[12] found that some insulin was absorbed in the presence of soybean trypsin inhibitor, diisopropylfluorophosphate, or indole-3-acetate. Furthermore, the latter investigators found some insulin was absorbed by everted sacs of rat intestine.

Although there is no doubt that the adult animal can absorb intact protein molecules, the amount appears to be extremely small. It is not known whether this absorption is due to transient local defects in the intestinal epithelium[6] or represents a more organized process. Perhaps a remnant of the fetal and newborn mechanism remains.

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CHAPTER 11

Vitamins

WATER-SOLUBLE VITAMINS

The water-soluble group of vitamins contains members of widely different chemical structures (Figure 98). If these compounds were all absorbed by simple diffusion one might make certain predictions as to the relative rates of absorption. It was pointed out in Chapter 2 that un-ionized compounds, weak acids, and weak bases, especially those of low molecular weight, are readily absorbed, while strong electrolytes are poorly absorbed. Applying this principle, the low molecular weight compounds such as nicotinamide, inositol, p-aminobenzoic acid, biotin, and ascorbic acid should diffuse readily while highly charged compounds such as thiamine and choline should not. Folic acid contains two carboxyl groups and might be considered in an intermediate position. The extreme case is vitamin B_{12}, which is both large (molecular weight about 1500) and ionized. One would predict that it could not be absorbed at all.

These predictions agree moderately well with the actual capacity of the intestine to absorb these compounds, with two striking exceptions (choline and vitamin B_{12}) which will be discussed later. The maximum absorptive capacity of the human small intestine for most of the water soluble vitamins of low molecular weight is of the order of 0.1 to 1 gm or more per day[32] (Table 51). In contrast, the rather large and highly basic thiamine molecule is very poorly absorbed, 5 mg. per day being

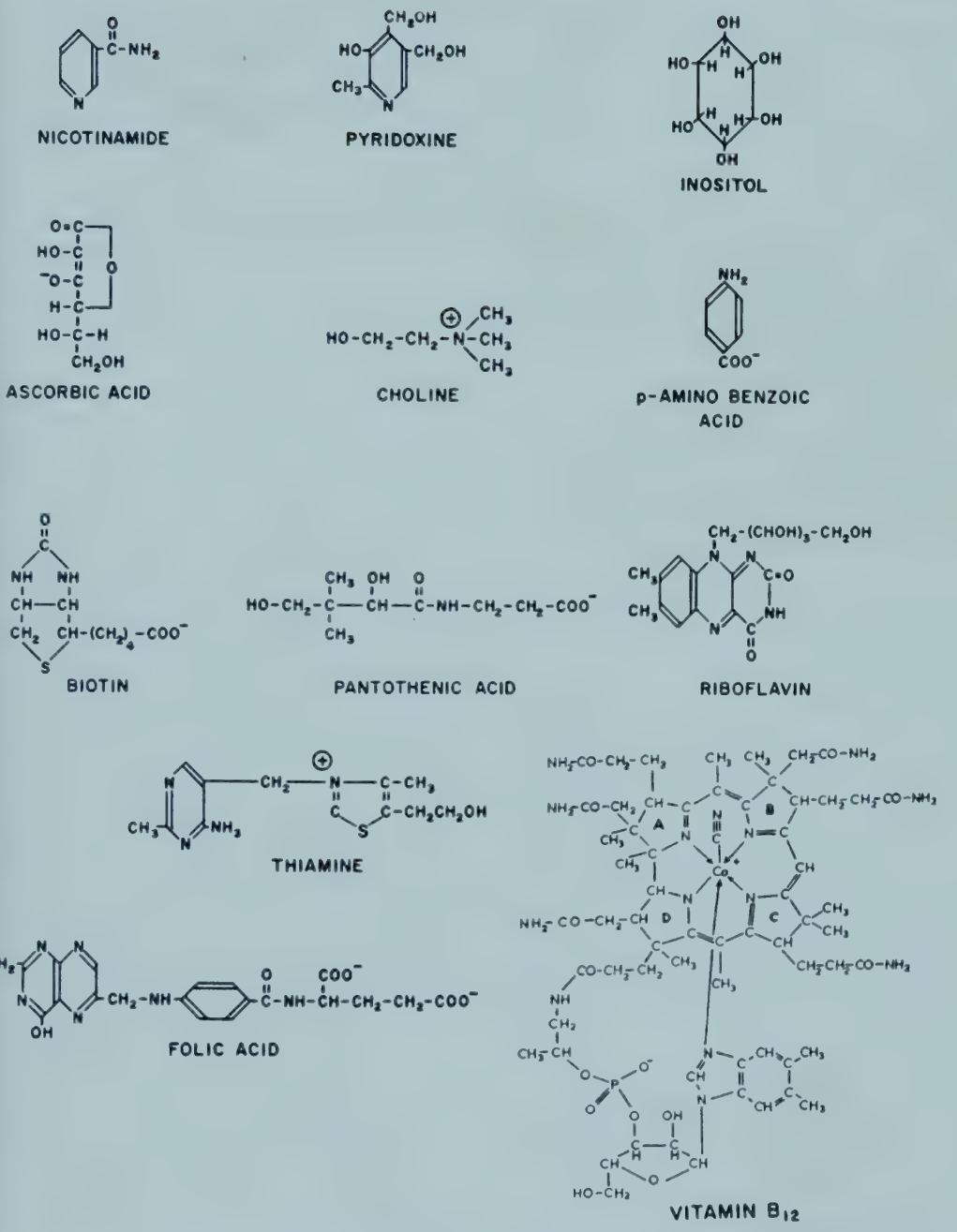


Figure 98. Water-soluble vitamins. The plus sign contained within a circle indicates the group is a strong base.

the maximum oral intake for human subjects without loss in the feces.[27] This is in agreement with the finding that the strongly basic tetraethylammonium ion is very poorly absorbed in the rat.[70] It is therefore difficult to explain why choline, which is also a strong base, is fairly well absorbed.[68] This suggests an active transport system for choline in the small intestine like that known to occur in the renal tubule.[65a] Although attempts to demonstrate active transport of choline in isolated hamster

Table 51. Absorption of Vitamins in Normal Human Adults

VITAMIN	ABSORPTION CAPACITY MOLES/DAY	DAILY REQUIREMENT MOLES
Nicotinamide	$> 1 \times 10^{-2}$*	1×10^{-4}
Ascorbic acid	$> 1 \times 10^{-2}$*	5×10^{-4}
Thiamine	2×10^{-5}	5×10^{-6}
Vitamin B_{12}	1×10^{-9}	5×10^{-10}†

*No published data found on maximum absorptive capacity but these values are probably much too low.

†This value is only a rough estimate.

Most of the data were obtained from Goodman and Gilman: The Pharmacological Basis of Therapeutics.[32]

intestine have thus far been unsuccessful,[88] further work on other animals is indicated.

So little quantitative information is available on absorption of most vitamins that no definite description of the mechanism can be given. In one of the few quantitative studies[56] urinary excretion of thiamine and riboflavin were taken as a measure of absorption. Although there is some uncertainty involved in such an assumption, the results are of interest. Figure 99 shows that urinary excretion of thiamine does not increase much within the oral dose range of 4 to 20 mg. The inference is that simple diffusion is not responsible for absorption but that some mechanism saturated at low levels is involved. In contrast to the results with thiamine, riboflavin excretion is linear over the dose range of 1 to 20 mg. This may indicate either that a special process is not yet saturated at the 20 mg. dose level or that riboflavin is absorbed by simple diffusion. The diffusion hypothesis is supported by recent *in vitro* studies.[73a,84] Spencer and Zamcheck[73a] found that the flux rates of riboflavin across the intes-

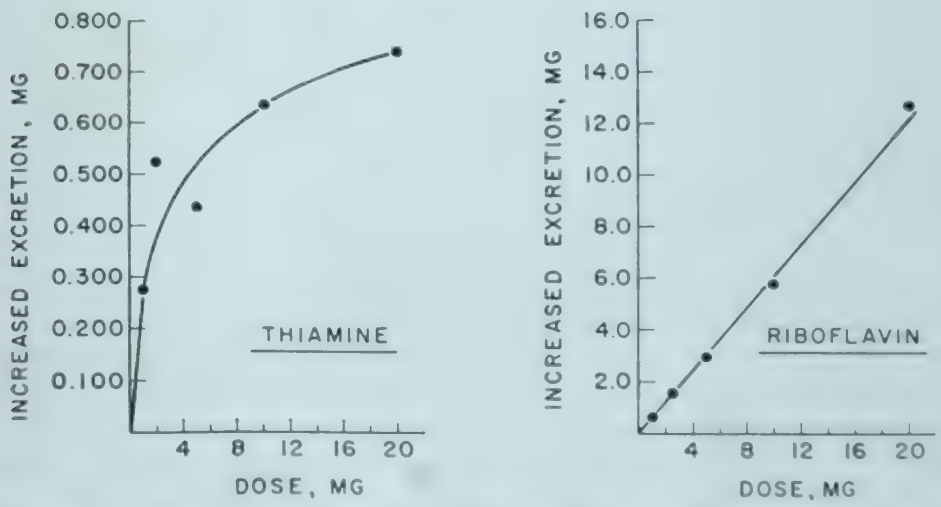


Figure 99. Effect of oral dose level on urinary excretion of thiamine and riboflavin in human subjects. (Morrison and Campbell: J. Nutrition, *72*, 1960.)

Table 52. Unidirectional Movement of Riboflavin Across Rat Intestine *in vitro*

Time of Incubation	Riboflavin Movement Across Intestinal Wall (μmoles/gm. tissue)	
	Mucosal to Serosal	Serosal to Mucosal
30 minutes	0.093 ($\pm$0.036)	0.079 ($\pm$0.059)
60 minutes	0.191 ($\pm$0.051)	0.151 ($\pm$0.099)

Sacs of rat intestine were incubated with riboflavin (4×10^{-4}M) initially on only one side. Each value represents average of 10 to 22 sacs ($\pm$ standard deviation). Modified from Spencer and Zamcheck: Gastroenterology, *40*:794, 1961.

tinal wall were the same in each direction (Table 52). Furthermore, no transport against a concentration gradient could be obtained with the intestine of the rat or hamster. Additional evidence was obtained by Muto,[57, 58] who found that only a small amount of riboflavin passed across the rat gut *in vitro* and that this movement was not inhibited by dinitrophenol.

One of the few *in vitro* studies of water-soluble vitamins is that of Turner.[84] In this elegant study, an attempt was made to use concentrations of vitamins which might be found in the lumen of the intestine *in vivo*. For this purpose highly sensitive and specific microbiological methods were employed. Figure 100 shows that the rate of absorption of pantothenic acid by everted sacs of rat intestine was approximately linear in concentration. Similar results were obtained with other vitamins. A careful study was made to see whether the intestine was capable of transporting these B-vitamins across the intestinal wall against a concentration gradient. No transport was observed with any of the vitamins tested (biotin, pantothenic acid, nicotinic acid, riboflavin, thiamine, folic acid, and vitamin B_{12}). Additional studies showed that the unidirectional flux rates of vitamins across the wall in opposite directions were similar (Table 53). All of these data are consistent with the view that these substances pass across the intestinal wall by simple diffusion. The stimulation of absorption of vitamin B_{12} by gastric intrinsic factor will be discussed in the next section.

Table 53. Unidirectional Flux Rates of Vitamins Across Everted Sacs of Rat Intestine

Vitamin	Conc.	Rate of Movement (10^{-12} moles)*	
		Mucosal to Serosal	Serosal to Mucosal
Biotin	2×10^{-6}M.	248	207
Pantothenic acid	2×10^{-5}M.	2,700	1,900
Vitamin B_{12}	1×10^{-7}M.	5.8	9.9

*Results expressed as 10^{-12} moles moved across the intestinal wall/3 cm. sac/hr. (Taken from Turner, J. B.: Thesis, University of Oxford, 1959.)

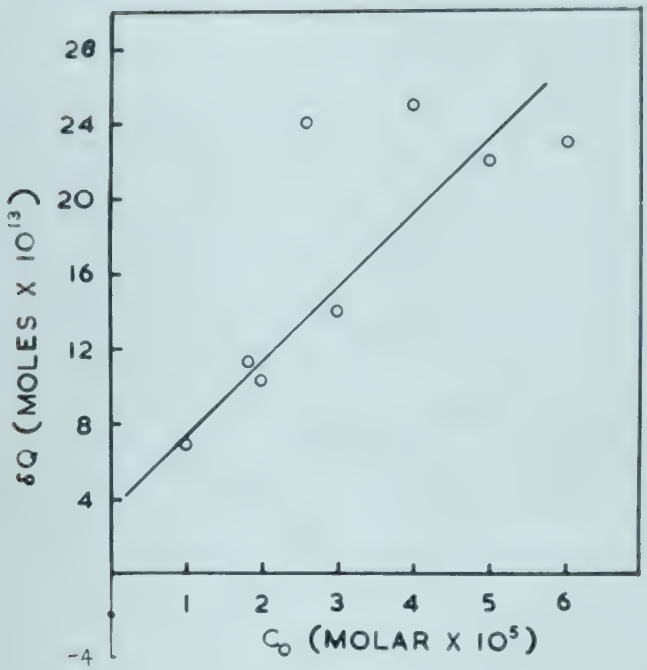


Figure 100. Absorption of pantothenic acid from the mucosal solution of everted sacs of rat intestine. δQ equals pantothenic acid absorbed by a 3 cm. sac per hour. C_0 equals initial concentration on mucosal side. (Turner: Thesis, University of Oxford, Queen's College, 1959.)

Vitamin B_{12}

The study of a human disease (pernicious anemia) was responsible for the discovery of the complex (and still incompletely understood) mechanism of the intestinal absorption of vitamin B_{12}. A primary defect in this disease is the inability of the stomach to produce a specific protein essential for normal absorption of the vitamin. This is the only case known in which the intestinal absorption of a large molecule requires the presence of an even larger molecule. This unusual requirement for a specific protein strongly suggests that the mechanism of absorption is entirely different from other recognized mechanisms of intestinal absorption.

In 1929, Castle[10] reported the first of a series of now-classical experiments on the etiology of pernicious anemia. Stimulated by the discovery of the liver treatment for pernicious anemia by Minot and Murphy,[55] and impressed by the achlorhydria regularly observed in these patients, he turned his attention to the stomach as the possible site of the primary lesion. Figure 101 shows the results of one of the original experiments in which remission was produced in a patient with pernicious anemia by daily administration of the contents of a normal human stomach recovered after the ingestion of 300 gm. of beef muscle. A few days after treatment was begun, there was a marked rise in the circulating reticulocytes. This was followed by a slow rise in the total circulating erythrocytes over a period of a few months. The hematological effect of feeding gastric contents from a normal individual is similar to that obtained

with Minot and Murphy's liver treatment (Figure 101). This and other experiments[12, 13] prompted Castle to postulate an "extrinsic factor" in the food which combined with some heat-labile "intrinsic factor" of normal gastric juice to produce a substance necessary for red cell production.

Extrinsic Factor. Nineteen years of intensive research by hundreds of workers in many countries were required for the final isolation and purification of the substance responsible for extrinsic factor activity Early work depended upon pernicious anemia patients to test the activity of purified factors in liver and muscle. Subsequently it was found that extrinsic factor was identical or similar to the growth factor in experimental animals and microorganisms and these assay procedures made possible the purification of the active substance. In 1948 vitamin B_{12} was obtained in pure form for the first time by two groups of workers.[67, 73] It was immediately tested by Castle's group[3] and the Mayo Clinic group[34] and found to be active in pernicious anemia patients either given parenterally or fed in the presence of intrinsic factor. This completed the identification of extrinsic factor as vitamin B_{12}.

Intrinsic Factor (I.F.): Following Castle's discovery of this heat-labile substance, the long and tedious process of purification began. Now, 32 years later, great strides have been made in purification, but a completely homogenous preparation has not yet been obtained. Although it is quite certain that I.F. is a protein, the presence or absence of a carbohydrate moiety in the molecule is not completely settled.

1. *Assay Methods.* The original assay method used by Castle is today still one of the most reliable procedures. This method consists of the daily feeding to a pernicious anemia patient (in relapse) the material

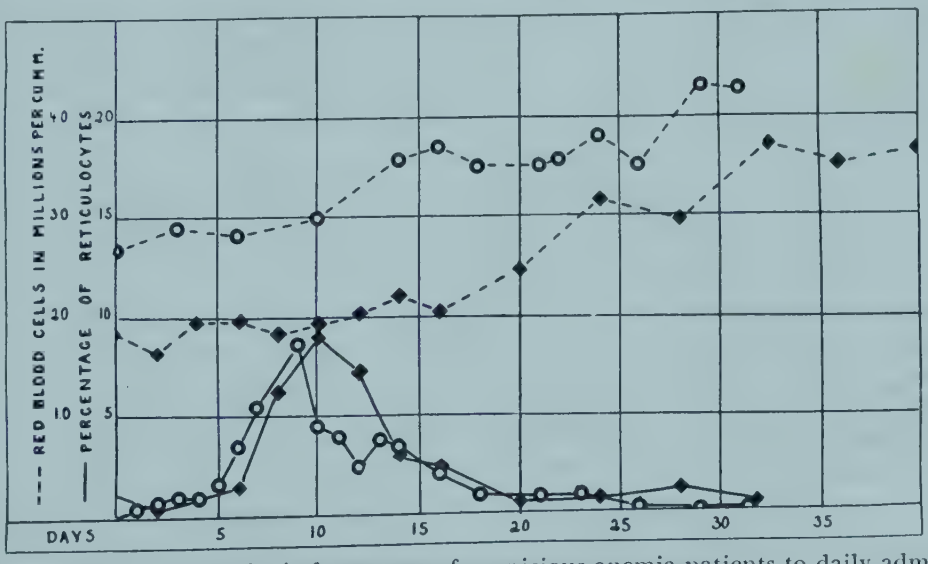


Figure 101. Hematological response of pernicious anemia patients to daily administration of liver (circles) or normal human gastric contents (diamonds). Dotted lines indicate red blood cell count; solid lines indicate reticulocyte count. (Castle: Am. J. M. Sc., *178*, 1929.)

to be tested for I.F. activity along with a source of vitamin B_{12}. If the reticulocyte count in the peripheral blood rises from less than 1 per cent to a value of about 5 per cent in five to ten days, intrinsic factor was present in the test material. Quantitative results are sometimes difficult to obtain and a refinement was therefore introduced by Minot and Castle[54] in which two test periods, separated by two weeks or more, were used to study the reticulocyte response of a standard I.F. preparation compared with an unknown preparation.

Following the availability of radioactive vitamin B_{12} in 1950[14] a variety of improvements in the assay was reported. In these newer methods absorption of radioactive vitamin B_{12} was determined indirectly by measurement of fecal[35, 86] or urinary excretion,[71] or by liver uptake.[29] Although all of these methods are quite reliable, they require a large number of cooperative pernicious anemia (or total gastrectomy) patients. An assay for human I.F. has been described using monkeys[44] and an assay of hog I.F. has been reported with gastrectomized hogs[36] but neither method is in general use.

Two *in vitro* assays have been developed. Miller and Hunter[53] and Herbert and London[39] showed that hog intrinsic factor stimulated B_{12} uptake by rat liver slices. This method has been utilized by a number of investigators and appears to be useful. The second method is the stimulation of B_{12} uptake with everted sacs of small intestine from a variety of animals.[38] Hog and human I.F. can be estimated by this method.[89] Boass and Wilson[6] have recently developed a simplified intestinal assay system involving small segments of tissue rather than sacs. These intestinal assay systems may be useful in future studies with I.F. as they appear to be specific for intrinsic factor, and with the use of the intestine from various animals a certain degree of discrimination among preparations of I.F. derived from different species may be perceived.

2. *Purification.* The history of the purification of gastric intrinsic factor and other B_{12}-binding substances was reviewed by Wijmenga in 1957.[87] More recent advances in purification have been reported by Latner and Merrills,[46] Jacob et al.[42] and Ellenbogen and Williams.[26] It has been emphasized by all these workers that preparations, though very highly purified, still contain varying amounts of contaminating material. There is now general agreement that an important property of this protein is its capacity to bind vitamin B_{12} firmly. Most preparations of I.F. have significant quantities of hexosamine and hexose, which would place I.F. in the group of substances known as mucoproteins or mucoids. Definitive chemical characterization must await further purification, which may come in the next few years.

3. *Site of I.F. Production in the Stomach.* There has been considerable difference of opinion as to the site of I.F. production. Only one study

will be mentioned here. Keuning et al.[45] showed with rat stomach that the fundus, which contains most of the "chief" cells, possesses most of the intrinsic factor activity, while the pyloric region contains little or none. Furthermore, these authors localized, histologically, the binding of B_{12} by an autoradiographic technique. Frozen sections of rat stomach were incubated for three hours with Co^{56}-vitamin B_{12} followed by careful washing and exposure to photographic film. The autoradiograph indicated darkening of the film in the areas of tissue that contained the most chief cells. Clearly, most of the B_{12}-binding material was in the chief or pepsinogen cells. The two pieces of evidence taken together suggest that I.F. is produced in the "chief" cells of rat stomach.

Site of B_{12} Absorption. The effect of location along the small intestine on B_{12} absorption varies somewhat with the species of animal. In man, a disease of the ileum (such as ileitis), but not of the duodenum or jejunum, is associated with malabsorption of vitamin B_{12}.[51, 21, 16] Furthermore, evidence obtained in normal individuals during laparotomy[9] indicates that the ileum is the normal site of B_{12} absorption. The ileum is also the site of absorption of B_{12} in the dog,[2] guinea pig,[89] and hamster.[89] In the hamster I.F. does not stimulate B_{12} uptake in everted sacs of jejunum[74] or colon[23] while it stimulates uptake up to fiftyfold in the lower ileum. In the rat, on the other hand, the midportion of the intestine is the site of maximum absorption of the vitamin in the presence of I.F.[7, 66, 74]

Species Difference in B_{12} Absorption. As early as 1929 hog intrinsic factor was found to be effective in patients with pernicious anemia, and as a result species differences were not explored until many years later. Coates et al.[20] were among the first to recognize clear species differences. They demonstrated that hog I.F. inhibited B_{12} absorption in both the rat and chick maintained on B_{12}-deficient diets. Furthermore, chick-stomach extract—B_{12} complex was active in promoting growth in B_{12}-deficient rats and chicks, while rat stomach extract inhibited B_{12} absorption in deficient chicks. It is presumed, but has not been established experimentally, that the chick possesses an intrinsic factor mechanism for the absorption of B_{12}. In the gastrectomized rat gastric juice or gastric mucosa of the same species stimulated B_{12} absorption[15, 18, 19, 40, 41, 59, 60, 85, 79] while both hog [18, 19, 38, 41, 59, 60, 69, 15] and human I.F. [18, 19, 59, 60] were ineffective. The human responds to I.F. from human,[10] rat,[1, 43, 79] and hog[76] but not from dog.[43] The monkey responds to human I.F.[44] *In vitro* methods have made feasible the study of the effect of a number of I.F. preparations on the intestinal uptake of B_{12} in a variety of small laboratory animals. Wilson and Strauss[89] have shown that the rat intestine responds only to rat I.F., and not to the five other I.F. preparations tested (Figure 102). Guinea pig intestine, on the other hand, responds to

all preparations tested, including hog and human I.F. It is concluded that striking species differences occur both in the intrinsic factors and in the receptor sites in the intestinal epithelium.

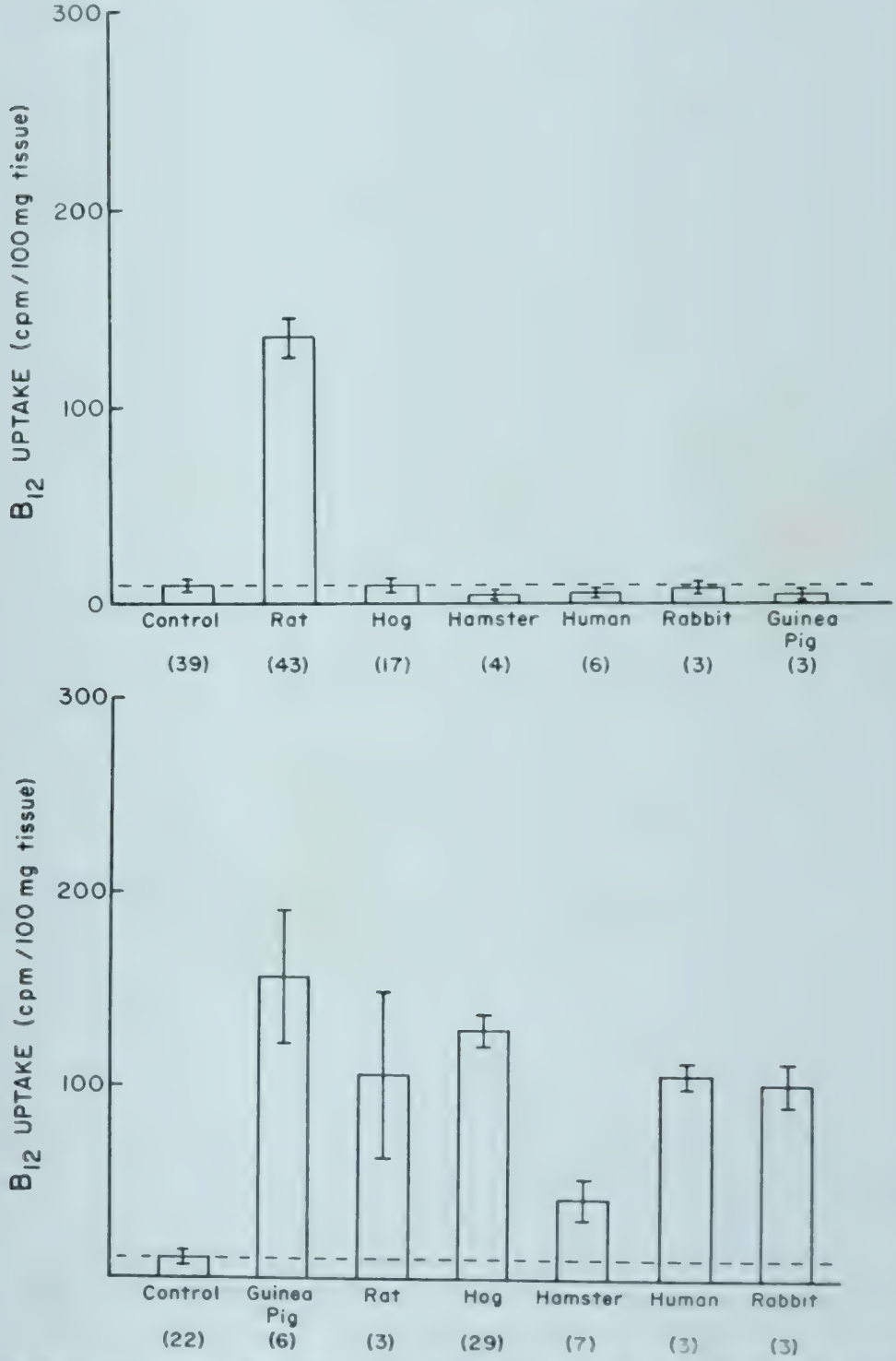


Figure 102. Stimulation of vitamin B_{12} uptake in sacs of intestine by intrinsic factor of different species. Above, rat intestine; Below, guinea pig intestine. The number of experiments indicated in parentheses. (Wilson and Strauss, Am. J. Physiol., *197*, 1959.)

Absorption of Intrinsic Factor. It would be extremely interesting to know the fate of intrinsic factor after its combination with B_{12} in the lumen of the intestine. One approach to this question has been the investigation of the possibility of absorption of the intact I.F. molecule. It has been known for many years that pernicious anemia patients ingesting hog I.F. over a period of time become refractory to treatment. During the past few years Schwartz,[72] Taylor,[77] and Lowenstein et al.[48] have presented evidence that patients receiving hog I.F. develop in their serums a substance which inhibits I.F. and is probably an antibody.[48] If antibodies are developed against hog I.F., intestinal absorption presumably occurred, although it is impossible to make quantitative estimates from the present data. Raney, Hansen, and Miller[65] have shown that after administration of 40 mg. of purified hog I.F. to rats, I.F. activity could be demonstrated in the blood. All of these experiments clearly show that some absorption occurs when relatively large amounts of I.F. from a different species are fed to rats or humans. Unfortunately these data do not answer the fundamental problem of whether I.F. is absorbed attached to B_{12} or in some other form during normal B_{12} absorption. The recent data of Cooper and Castle,[22] which will be referred to in more detail later, suggest that I.F. is removed from B_{12} when B_{12} passes through the epithelial cell.

Route of B_{12} Absorption. There is limited information, at present, on the route taken by B_{12} from the epithelial cell of the intestine to the blood. Taylor and French[78] have shown that only a small fraction of orally administered B_{12} appears in the thoracic duct lymph of the rat. During thoracic duct cannulation considerable B_{12} is found in the liver

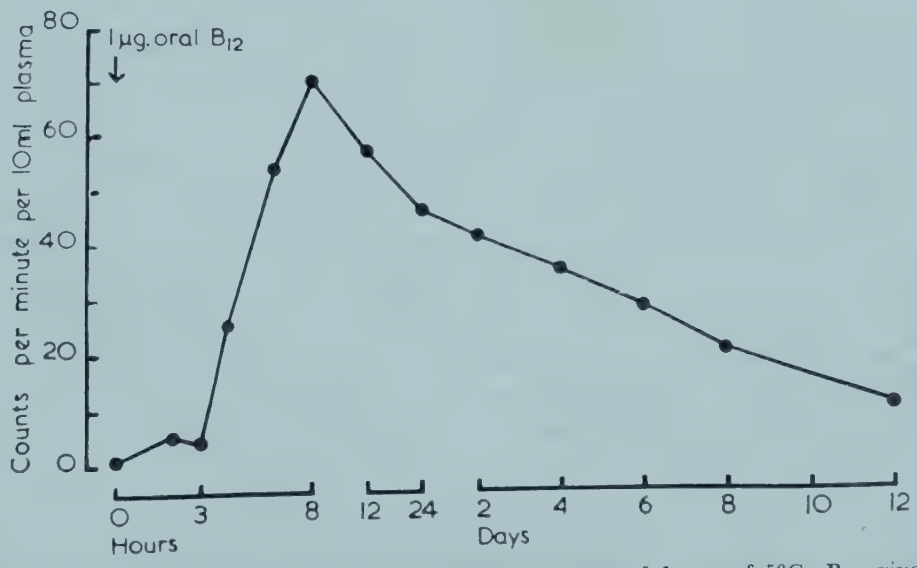


Figure 103. Plasma radioactivity after an oral dose of 1 µg. of ^{56}Co-B_{12} given to a normal subject. (Booth and Mollin: Brit. J. Haematol., *2*, 1956.)

and kidney, which suggests that most of the B_{12} is absorbed into the portal vein.

Delay in B_{12} Absorption. Following oral administration of physiologic doses of radioactive vitamin B_{12} to normal humans or rats, no radioactivity appears in the blood for one to four hours.[8, 7, 24, 25] After this lag, radioactivity increases in the blood for the next few hours, reaching a peak level about six to ten hours after administration (Figure 103). There appears to be little delay in stomach emptying time or transit in the lumen of the small intestine as large amounts of radioactivity were present in the intestinal wall in 15 minutes.[7] The failure of the peripheral blood level to rise in the first few hours might be due to efficient removal of the vitamin by the liver, followed by the saturation of the liver and the appearance of B_{12} in the blood. This possibility, however, has been effectively ruled out by the observation that for one to two hours after feeding no vitamin was found either in portal vein blood[25] or liver.[7] The one remaining conclusion is that one to four hours are required for the transit of vitamin B_{12} across the epithelial cell of the small intestine.

Mechanism of Absorption. A variety of hypotheses for the mechanism of action of intrinsic factor has been entertained in the past (see the review by Castle[11]) but only the more recent views will be considered here. It is now clear that gastric I.F. (in some form) stimulates the epithelial cells of the small intestine to absorb vitamin B_{12} (in either a free or a combined form). I.F. might stimulate the absorption of (a) many substances, (b) free B_{12}, or (c) a B_{12}-intrinsic factor complex. Possibility (a) would seem unlikely as the pernicious anemia patient does not seem to have any serious absorptive defect other than that of B_{12}. Gardner et al.[28] have specifically considered this possibility and conclude that I.F. does not affect absorption of glucose, tyrosine, casein, or folic acid. With intestinal sacs *in vitro* I.F. had no effect on glucose transport[74] or I^{131}-labeled albumin uptake.[90]

The question of whether B_{12} is absorbed free or bound to I.F. has been approached experimentally in three different laboratories.[5, 83, 60] The basic experiment was to feed to a gastrectomized or pernicious anemia patient two types of solutions: a mixture of radioactive B_{12} and cold B_{12}-I.F. complex, and a mixture of cold B_{12} and radioactive B_{12}-I.F. complex. If it was the B_{12}-I.F. complex that entered the cell, the second mixture would result in more absorption of radioactivity than the first; if free B_{12} was absorbed, the reverse would be true. Table 54 shows the average values obtained in nine experiments with eight total gastrectomy patients. When free B_{12} and B_{12}-I.F. complex are fed together, the complex is absorbed three times as rapidly as the free vitamin. This observation was confirmed by Nieweg, Shen, and Castle[60] in the

Table 54. The Importance of B_{12}-Binding in the Mechanism of Intrinsic Factor Action

Mixture fed by mouth	Per cent of oral dose of radioactive B_{12} appearing in the urine in 24 hours
Radioactive B_{12} plus nonradioactive B_{12}-I.F. complex	2.0
Nonradioactive B_{12} plus radioactive B_{12}-I.F. complex	6.4

Eight totally gastrectomized patients were fed first one mixture and then, days or weeks later, were fed the second mixture. Urinary excretion of radioactive B_{12}, as described by Schilling,[71] was used as the measure of vitamin B_{12} absorption. The first mixture consisted of 2.2 μg. Co^{60}-vitamin B_{12} followed immediately by a mixture of 2.2 μg. nonradioactive B_{12} in 50 ml. of pooled human gastric juice. The second mixture consisted of 2.2 μg nonradioactive B_{12} followed immediately by a mixture of 2.2 μg Co^{60}-vitamin B_{12} in 50 ml. of gastric juice. (Taken from Toporek: Am. J. Clin. Nutrition, *8*:297, 1960.)

rat. These data convincingly suggest that combination of B_{12} with intrinsic factor occurs prior to intestinal absorption. Purified preparations of I.F. usually bind very tightly with B_{12} and Cooper and Castle[22] have recently shown that this binding is so effective that B_{12} can be removed from its binding sites on other proteins of the diet by I.F.

There is evidence that neither B_{12} nor I.F. attaches to the epithelium prior to formation of the vitamin-I.F. complex[74, 75] (Table 55). Herbert[37] and Cooper and Castle[22] found that ethylenediaminetetraacetic acid (a chelating agent for divalent cations) inhibits B_{12} absorption in the presence of I.F. and concluded that a divalent ion, probably calcium, is essential for the attachment of the B_{12}-I.F. complex to the gut wall. However, the removal of calcium from the solution bathing the mucosal surface

Table 55. Effect of Preincubation of Rat Intestine in Intrinsic Factor on B_{12} Uptake

Sac no.	Addition to Krebs saline		B_{12} uptake by intestine, cpm./100 mg. tissue
	Period 1 (45 min.)	Period 2 (60 min.)	
1	No addition	B_{12}	21
2	No addition	B_{12} + I.F.	425
3	I.F.	B_{12}	25
4	I.F.	B_{12} + I.F.	495

Four sacs prepared from consecutive segments of midgut were preincubated (period 1) for 45 min. in either Krebs bicarbonate-saline alone or a similar solution containing 1 ml. rat gastric juice. Sacs removed from flask and washed carefully for 1 min. in calcium-containing Krebs bicarbonate-saline. Sacs then transferred to another flask and incubated for a second period (60 min.). Sacs removed and washed in three separate 100-ml. volumes of Krebs solution. (Taken from Strauss and Wilson: Am. J. Physiol., *198*:103, 1960.)

of the intestine had no more than slight effects.[37, 75] It may be argued that enough calcium is present on the cell surface or leaks out of the cell under these conditions to vitiate the experiment. The EDTA experiments, on the other hand, are open to criticism for, at least *in vitro*, this chelating agent removes the epithelial cells from the lamina propria (at least in the hamster) in an incubation of 15 to 30 minutes at 37 C. This effect can be observed with EDTA concentrations as low as 0.1 mM. (in the absence of added divalent cation). The role of calcium in binding B_{12}-I.F. complex to the epithelial cells needs further investigation.

An important recent observation has been made by Cooper and Castle[22] on the fate of B_{12} within the epithelial cell. These investigators found that homogenates of rat intestinal mucosa could remove B_{12} from its complex with rat I.F. but not if complexed with human or hog I.F. (Figure 104). Although this ability to break the bond between B_{12} and I.F. is not entirely lost when the extract is boiled, the phenomenon has properties suggestive of an enzymatic reaction and appears highly species-specific. The authors suggest that once the B_{12}-I.F. complex enters the cell the complex is broken, liberating free B_{12}. "The vitamin B_{12} thus

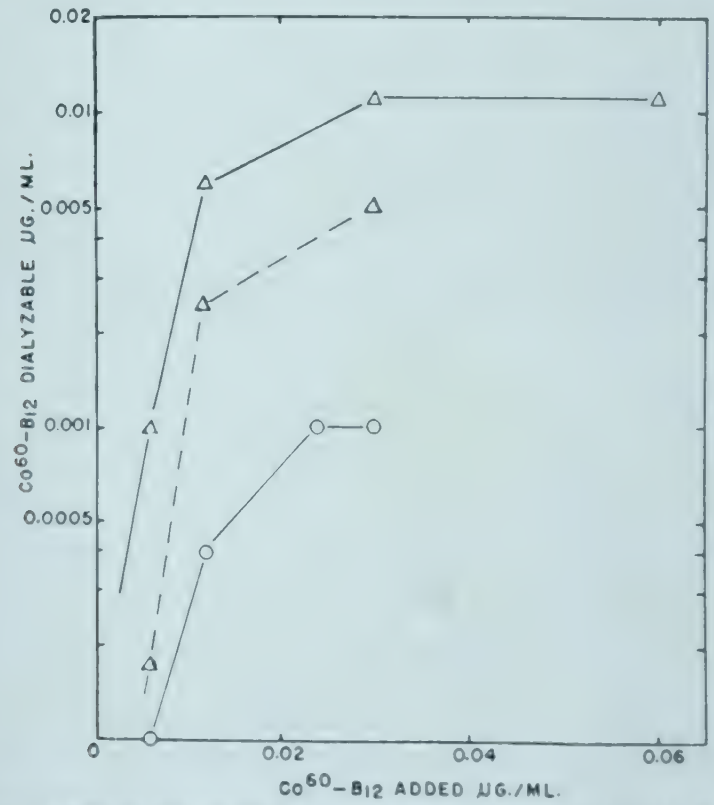


Figure 104. Effect of rat intestinal extract in rendering dialyzable Co^{60} B_{12} bound to rat stomach extract (triangles) and to human gastric juice (circles). The effect of previously boiled rat intestinal extract upon Co^{60} B_{12} bound to rat stomach extract is also shown (triangles connected by broken line). (Cooper and Castle, J. Clin. Invest., *39*, 1960.)

freed would then either be accepted by an appropriate transport mechanism or would simply diffuse passively into the blood vessels of the intestine."[22]

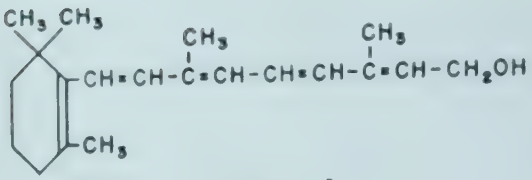
Recent studies on the I.F. stimulation of B_{12} uptake by rat visceral yolk sac might be mentioned. The yolk sac is an outgrowth of the midgut, to which it remains attached throughout fetal life. Furthermore, histologically it resembles intestine, villi lined with columnar epithelium possessing a brush border. Padykula and Wilson[63] have found that rat yolk sac possesses the I.F.-stimulated B_{12} absorption system, apparently similar to that of the adult gut. The striking observation was that intrinsic factor had its greatest stimulating effect at a stage of fetal development (13 days) at which pinocytosis (by criteria of electronmicroscopy) is most highly developed. Although this correlation may be fortuitous, it is possible that pinocytosis is involved in the cellular uptake of the B_{12}-I.F. complex. It might be pointed out that pinocytosis is the only mechanism known, at present, to be capable of translocating large protein molecules across cell membranes, especially of the gut.[17]

FAT-SOLUBLE VITAMINS

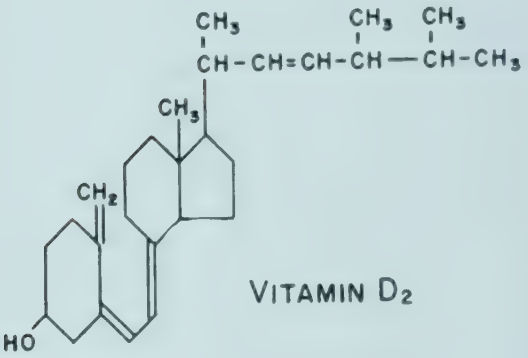
Because of their solubility in lipids, this group of compounds is usually associated with other lipids in the diet and perhaps absorbed by similar mechanisms (see Figure 105 for structures). Bile salts are essential for adequate absorption of vitamin K,[64] vitamin D,[33, 80] and carotene.[61] The other vitamins (A and E) may also require the presence of bile but the evidence is incomplete. This requirement for bile salts indicates that emulsification of these water-insoluble compounds is required prior to absorption. Although the mechanism of absorption of these compounds has not been studied in detail, diffusion through the cell membrane by means of solution in the lipid of the membrane seems reasonable. There is, to date, no evidence of any specialized transport system for these compounds.

Vitamin K

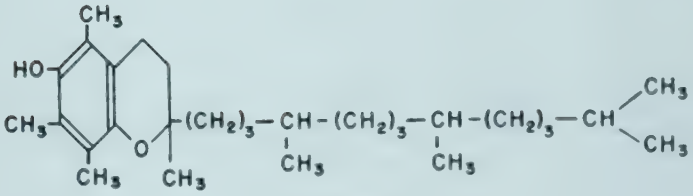
Some new synthetic compounds with vitamin K activity offer some interesting problems in intestinal absorption. Menadione, which is structurally similar to one portion of the naturally occurring vitamin, is insoluble in water and highly soluble in organic solvents and oils (Figure 106). This compound is biologically active and is extensively administered orally. It requires bile salts for absorption and, consequently, when given to patients with bile duct obstruction it must be accompanied by oral administration of bile salts. In these patients a



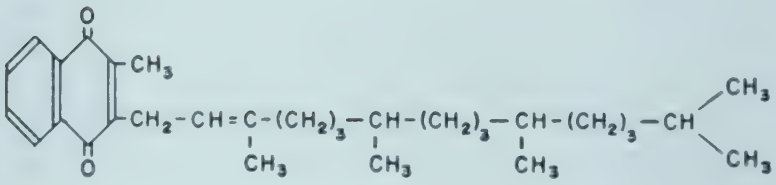
VITAMIN A_1



VITAMIN D_2



VITAMIN E



VITAMIN K_1

Figure 105. Fat-soluble vitamins.

water-soluble bisulfite derivative of menadione, which does not require bile salts for its absorption, is well absorbed.[32] This is an interesting example in which lipid solubility is a liability and conversion into a water-soluble form obviates the need for emulsification with bile salts.

An important source of vitamin K in many animals (including man) is the bacterial flora of the intestine. When the bacterial flora is altered by administration of massive doses of antibiotics, serious defi-

ciencies in vitamin K may result. This interesting problem has been reviewed by Mickelsen.[52]

Vitamin A

The normal daily requirement of this vitamin (about 2 mg. of the alcohol) is readily and completely absorbed by the intestine. The mechanism by which vitamin A is absorbed is not clear. It has been tacitly assumed that it is absorbed with lipids. Aqueous dispersions, however, are absorbed more quickly than similar quantities given in oil.

It has been known for over 30 years that carotene could replace vitamin A in the diet as a growth factor and that this replacement was due to the chemical conversion of carotene into the vitamin (see the review by Lowe and Morton[47]). Although it was first thought that the liver was the site of conversion, it is now known that the major site for this process after oral administration is the epithelial cell of the small intestine. This conclusion was based on experiments with rats deficient in vitamin A, performed almost simultaneously in three different laboratories.[49, 81, 30, 82, 31] A single dose of carotene was fed to rats and within 15 minutes vitamin A was detected in the intestinal mucosa, but in no other organ of the body. Vitamin A then appeared in the intestinal lymphatics and after an hour was found in both blood and liver. The intestine maintained a high level of the vitamin for at least four hours. During subsequent hours and days the vitamin A concentration in the intestine fell to almost zero while that in the liver was maintained at a high level. Olsen[62] has shown that the rate of conversion of carotene to

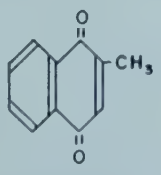
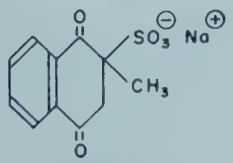
	SOLUBILITY	
	WATER	ETHER
 MENADIONE	0	+
 MENADIONE SODIUM BISULFITE	+	0

Figure 106. Two synthetic compounds with vitamin K activity.

vitamin A in washed rat intestinal loops *in situ* was five to ten times that required per day for this species.

It should be noted that in some species the intestine is not the sole site for this interconversion.[1, 62, 50] Conversion of intravenously injected carotene (emulsified in detergent) to vitamin A in the rat, was not affected by removal of the intestines.[4] Injections of carotene to calves,[50] however, did not give rise to vitamin A, suggesting that in this species conversion of carotene to the vitamin occurs in the intestine during absorption.

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CHAPTER 12

Drugs

Although drugs are commonly considered "foreign" substances the *Pharmacopeia* lists many compounds which are in the normal diet or are intermediates of tissue metabolism. A study of the intestinal absorption of this spectrum of substances, therefore, would draw on much of the information found in other chapters of this book. There are a few classes of compounds, however, foreign substances used specifically for the treatment of disease, which have not been covered in other chapters.

GENERAL PRINCIPLES

The epithelial cells of the intestine, like other cells, behave as though they were surrounded by a plasma membrane with the properties of both proteins and lipids, with occasional small water-filled channels which penetrate the membrane. More than 60 years ago Overton[21] pointed out that the rate of entrance of many substances into cells could best be explained by solution of the substance in the lipid parts of the cell membrane followed by diffusion into the interior of the cell. This principle has become one of the cornerstones of cellular physiology. Many illustrations will be given in which the lipid route is the most important means of drug absorption. To possess properties of lipid solubility a substance must contain nonpolar groups such as long-chain

alkyl groups and aromatic rings, and must contain a minimum of hydrophylic groups such as hydroxyl, aldehyde, ketone, or ionizable carboxyl or amino groups. It should be noted that in a molecule with both polar and nonpolar properties, one or the other aspect may predominate. Thus, phosphatidic acid, which possesses an ionizable phosphate group and two long-chain glyceride groups, is soluble in ether as a sodium salt.

The second route for diffusion across a cell membrane is by means of the water-filled channels. There is now evidence that the average pore radius in the intestinal epithelial cell is of the order of 4 Å (see Chapter 3, p. 44). This, of course, is only the estimate for the average pore size and there may well be a few with very much larger radii.

Another route of absorption available for drugs is the large series of active transport systems in the epithelial cell for absorption of sugars, amino acids, ions, etc. Two foreign pyrimidines with antitumor activity are actively transported[27] by the pyrimidine transport system discovered by Schanker and Tocco.[30] The nonmetabolizable sugar derivative, 3-0-methylglucose, which was once considered as a possible sweetening agent for diabetics, is actively transported[36] by the sugar transport system.

When considering drug absorption from the clinical point of view slow continuous absorption may often produce much more desirable effects than very rapid absorption. This is particularly true in the case of antibacterial agents and other substances where a maintained blood level is desirable. As much of the available data on absorption is given in terms of clinical effectiveness or blood level, the term "well absorbed" or even "rapidly absorbed" must be evaluated in terms of these points of reference. An example of ambiguity with regard to the term "well absorbed" may be given for the simple ion, inorganic phosphate. Although this ion is usually thought of as "well absorbed," the capacity of the intestine to absorb it is so slight that phosphate (as a salt) in a 5 to 10 gm. dosage is an effective cathartic.

THE EFFECT OF pH ON THE ABSORPTION OF WEAK ELECTROLYTES

The intestinal mucosa is relatively impermeable to charged compounds, especially if they are of large molecular weight. Strong acids and strong bases, which exist almost entirely in the ionized form at body pH, are generally poorly absorbed. Weak electrolytes, however, exist in solution in both ionized and un-ionized form, the proportion of the two depending on the pK of the ionizable group and the pH of the solution. The un-ionized species, especially if they are lipid soluble, will cross

cell membranes with relative ease.[12] As the uncharged molecules continue to diffuse into the cell the equilibrium between the two species outside the cell is upset, resulting in the formation of more uncharged molecules. In the intestine this process can continue until all of the substance is absorbed, provided its concentration at the base of the epithelial cell is reduced to a low level by efficient circulation of blood. The rate of absorption will depend upon the concentration difference of the un-ionized species across the cell and the permeability constant. Blood flow is probably seldom rate-limiting in these conditions.

Schanker et al.[29] have recently made a careful study of the rate of absorption of a variety of drugs with different pK_a values. The relation between pK_a and rate of absorption of acids and bases is shown in Figure 107. The lowest pK_a of an acidic drug consistent with rapid absorption was about 3, while the corresponding highest pK_a for basic drugs was 8. If one calculates the fraction of un-ionized to ionized molecules which exists for compounds with these pK_a values at pH 6.6, one obtains a value of 1/6000 for the acid and 1/16 for base. As it seems unlikely that un-ionized acids are very much more diffusible than un-ionized bases, Schanker et al.[29] have concluded that "effective" pH at the cell membrane must be lower. If the "effective" pH were 5.3 the ratio of un-ionized to ionized drug necessary for rapid absorption would be 1/300 for both an acid of pK_a 2.8 and a base of pK_a 7.8. This hypothesis, curious as it may seem at first sight, seems more plausible than the alternative hypothesis of entirely different diffusion properties of uncharged acids and bases.[24, 25, 26]

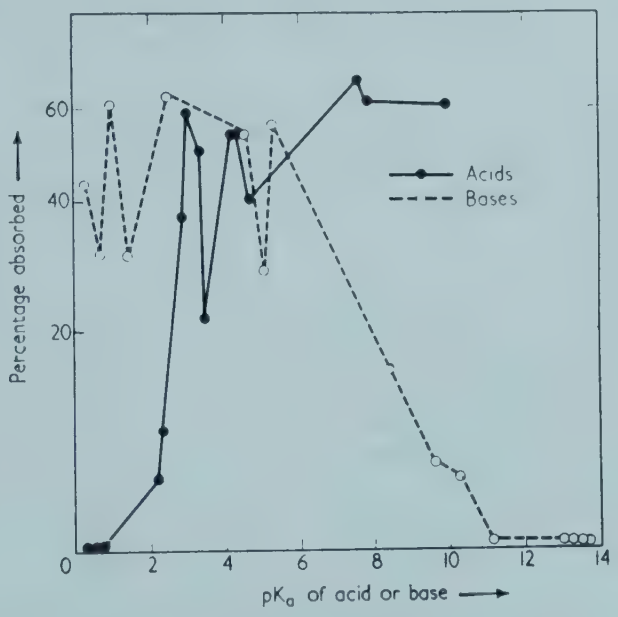


Figure 107. Comparison between pK_a and intestinal absorption of drugs in the rat. (Schanker, J. Med. & Pharmacol. Chem., *2*, 1960.)

Stomach. In 1940 Travell[33] showed that pH has a marked effect on the absorption of the weak base, strychnine, from the stomach of the cat. When strychnine in acid solution was injected into the tied stomach it had no effect on the animal; when injected in alkaline solution it rapidly caused symptoms of toxicity and the animal died in relatively few minutes. Table 56 shows the effect of pH on the toxicity of this drug. A careful study of the movement of drugs across the gastric mucosa was performed by the Bethesda group, in dogs,[31] rats,[28] and man.[10] In one study, Shore et al.[31] administered drugs to dogs with Heidenhain gastric pouches and determined the concentration in both blood and gastric juice. A group of weak bases, which were partly un-ionized in plasma, moved across the gastric mucosa and appeared in gastric juice in high concentration. This demonstrates, with drugs, what had been shown with dyes, that basic compounds are "secreted" into gastric juice (see Höber[7]). This process is the result, not of any active transport process, but of the characteristic permeability of the membrane to uncharged species and the pH difference across the membrane. The molecules are effectively "trapped" in gastric juice in the ionized form (see Chapter 3). It might be mentioned at this point that weak acids appear in high concentrations in alkaline secretions of the pancreas, liver and kidney.[7, 32, 22]

Table 56. Effect of pH on rate of Absorption of Strychnine from the Stomach

pH of solution in the stomach	Percentage of strychnine in the undissociated form	Time of death following injection into the stomach (minutes)
8.0	54.0	24
6.0	1.2	83
5.0	0.1	150
3.0	0.001	survived

Strychnine sulfate (5 mg.) was injected into the stomach of an anesthetized cat. Modified from Travell: J. Pharmacol. & Exper. Therap., *69*:21, 1940.

In addition, these workers investigated the absorption of drugs introduced into the ligated rat stomach.[28] It was found that acids with a pK_a of 2 or more were well absorbed. Weak bases, on the other hand, were absorbed only if the bases were extremely weak (pK_a of 5 or less). In man a similar pattern was found: The weak acids acetylsalicylic acid, thiopental, and secobarbital were readily absorbed while the bases quinine, ephedrine, and aminopyrine were not. These weak acids were absorbed even faster than ethanol, a substance usually quoted as being the most readily absorbed substance in the stomach.

Small Intestine. Schanker et al.[29] have made a careful study of drug absorption from the small intestine. Substances with a pK_a between 2 and 9 were well absorbed while strong acids and bases were not (Tables 57 and 58). If the "effective" pH at the cell wall were 5 (as postulated by these workers) the minimal ratio of un-ionized to ionized for effective absorption would be between 1:100 and 1:1000. As one would anticipate, alteration of luminal pH has a profound effect on the absorption rate of weak electrolytes. They found that the absorption rate of quinine (pK_a of 8.4) was much more rapid at pH 8 than at pH 5. With the weak acid nitrosalicylic acid (pK_a of 2.3) there was no absorption at pH 7, but considerable absorption at pH 5 (see Figure 26, p. 46).

EFFECT OF LIPID SOLUBILITY ON ABSORPTION RATE

Since the work of Overton[21] it has been known that lipid solubility is an important factor in determining the rate of penetration of substances across cell membranes. Höber and Höber[8] in 1937 provided evidence that the rate of absorption of certain compounds is related to lipid solubility. They showed that malonamide, because of its greater lipid solubility, is absorbed more rapidly than lactamide. An elegant example of this phenomenon is taken from the work of Schanker[24] on the absorption of different barbituric acid derivatives. Increasing the length of the alkyl side chain of the molecule produces compounds which are

Table 57. Absorption of Organic Acids by the Rat Small Intestine

Acid	pK_a	Per cent absorbed	
		Actual	Relative to aniline
5-Sulfosalicylic	(strong)	2 (2)	2
Phenol red	(strong)	2 (4)	2
Bromphenol blue	(strong)	2 (2)	2
o-Nitrobenzoic	2.2	5 ± 2 (2)	5
5-Nitrosalicylic	2.3	9 ± 2 (3)	9
Tromexan	2.9	35 ± 7 (3)	37
Salicylic	3.0	60	—
m-Nitrobenzoic	3.4	54 ± 0 (2)	50
Acetylsalicylic	3.5	20 ± 4 (6)	21
Benzoic	4.2	51 ± 5 (2)	54
Phenylbutazone	4.4	65 ± 7 (3)	54
Acetic	4.7	42 ± 1 (3)	40
Thiopental	7.6	55 ± 6 (3)	67
Barbital	7.8	30 ± 4 (2)	25
p-Hydroxypropiophenone	7.8	61 ± 9 (5)	61
Phenol	9.9	51 ± 8 (3)	60

The per cent absorbed is expressed as the mean ± the range, followed by the number of experiments in parentheses. Taken from Schanker, Tocco, Brodie, and Hogben: J. Pharmacol. & Exper. Therap., *123*:81, 1958.

Table 58. Absorption of Organic Bases by the Rat Small Intestine

BASE	pK_a	PER CENT ABSORBED	
		ACTUAL	RELATIVE TO SALICYLIC ACID
Acetanilide	0.3	42 ± 5 (2)	43
Theophylline	0.7	29 ± 1 (3)	30
p-Nitroaniline	1.0	68 ± 7 (2)	61
Antipyrine	1.4	32 ± 6 (3)	30
m-Nitroaniline	2.5	77 ± 2 (2)	63
Aniline	4.6	54	—
Aminopyrine	5.0	33 ± 4 (4)	27
p-Toluidine	5.3	59 ± 3 (3)	56
Quinine	8.4	15 ± 2 (6)	15
Ephedrine	9.6	7 ± 3 (2)	6
Tolazoline	10.3	6 ± 1 (2)	5
Mecamylamine	11.2	< 2 (2)	< 2
Darstine	(strong)	< 2 (2)	< 2
Procaine amide ethobromide	(strong)	< 2 (2)	< 2
Tetraethylammonium	(strong)	< 2 (2)	< 2
Tensilon	(strong)	< 2 (2)	< 2

The per cent absorbed is expressed as the mean ± the range, followed by the number of experiments in parentheses. Taken from Schanker, Tocco, Brodie, and Hogben: J. Pharmacol. & Exper. Therap., *123*:81, 1958.

more readily absorbed by the intestine (Figure 24, p. 45). Lipid solubility and not molecular size is clearly the determining factor in permeability. Hogben et al.[11] (Table 59) have listed compounds in the order of their solubility in organic solvents. There is a rough agreement between lipid solubility and absorption rate.

SALINE CATHARTICS

The physiologic basis for the action of saline cathartics is the very slow rate of intestinal absorption of these ions and their excretion with an osmotic equivalent of water. For example, if none of a 9 gm. dose of magnesium sulfate were absorbed it would be excreted in a volume of about 500 ml., as an approximately iso-osmotic solution. The desirable properties of a good saline cathartic are (a) nontoxic, (b) poorly absorbed, and (c) low molecular weight (so that maximum osmotic activity is obtained per gm.).

The compounds shown in Figure 108, with the exception of fumarate, are all in common use as saline cathartics.[6] Divalent anions and cations are very poorly absorbed by the gastrointestinal tract although small amounts do appear in the blood and urine. Presumably, divalent ions are too large to pass readily through the water-filled channels of the cell membrane and because of their highly polar properties are insoluble in the lipid of the membrane. Phosphate ion, which is an important nutrient, is absorbed slowly enough to make it an effective

Table 59. Comparison Between Intestinal Absorption and Lipid: Water Partition of the Un-ionized Forms of Organic Acids and Bases

DRUG	PER CENT ABSORBED	KHEPTANE	KCHLOROFORM
Phenylbutazone	54	> 100	> 100
Thiopental	67	3.30	> 100
p-Toluidine	56	3.26	97.5
Aniline	54	1.10	26.4
m-Nitroaniline	63	0.24	39.2
Benzoic acid	54	0.19	2.9
Phenol	60	0.15	2.3
p-Nitroaniline	61	0.13	19.8
p-Hydroxypropiophenone	61	0.12	5.1
Salicylic acid	60	0.12	2.9
m-Nitrobenzoic acid	50	0.06	2.6
Aminopyrine	27	0.21	> 100
Acetylsalicylic acid	21	0.03	2.0
Acetanilide	43	0.02	7.6
Theophylline	30	0.02	0.3
Antipyrine	30	0.005	21.2
Barbital	25	< 0.002	0.7
Theobromine	22	< 0.002	0.4
Sulfanilamide	24	< 0.002	0.03
p-Hydroxybenzoic acid	23	< 0.002	0.01
Barbituric acid	5	< 0.002	0.008
Sulfaguanidine	< 2	< 0.002	< 0.002
Mannitol	< 2	< 0.002	< 0.002

Drugs were distributed between an organic solvent and an aqueous phase whose pH was such that the drug was largely in the un-ionized form. Taken from Hogben, Tocco, Brodie, and Schanker: J. Pharmacol. & Exper. Therap., *125*:275, 1959.

cathartic. At the pH of the jejunum (between 6 and 7) phosphate would exist as a mixture of monovalent and divalent species. Since very little would exist in the un-ionized form, it could not be absorbed by the lipid route, and its size probably would prevent much from entering by the pore route. It is uncertain, therefore, how phosphate ion is absorbed. Perhaps it is by some carrier mechanism.

The two dicarboxylic acids, tartaric acid and fumaric acid, are very poorly absorbed. At pH 7, about one molecule out of 1,000 is monovalent and 1 out of 1,000,000 is un-ionized. It is interesting that fumaric acid, a normal intermediate of metabolism, cannot be absorbed appreciably by the intestine.[5]

A monocarboxylic acid whose un-ionized form is lipid-insoluble is poorly absorbed. Gluconic acid in doses of 10 gm. or more is an effective cathartic.[4]

SULFONAMIDES

Few quantitative studies are available on intestinal absorption of sulfonamides. Most studies deal with the parameters most interesting

ION	% UN-IONIZED AT pH 7
Mg^{++}	0
$SO_4^{=}$	0
$HPO_4^{=}$	0.005
$^{-}O-\overset{O}{\overset{\|}{C}}-\overset{OH}{\overset{\|}{C}}H-\overset{OH}{\overset{\|}{C}}H-\overset{O}{\overset{\|}{C}}-O^{-}$	0.00001
$^{-}O-\overset{O}{\overset{\|}{C}}-CH=CH-\overset{O}{\overset{\|}{C}}-O^{-}$	0.00001

Figure 108. Saline cathartics and ionization. Compounds listed are magnesium, sulfate, phosphate, tartrate, and fumarate.

to the clinical investigator (e.g., blood level and urinary excretion). Although these studies are valuable from the clinical point of view, they do not give a clear quantitative picture of absorption, as so many variables are involved, such as chemical alteration within the body and excretion. Hogben, Tocco, Brodie, and Schanker[11] have performed quantitative studies on two members of the sulfonamide family, sulfanilamide and sulfaguanidine. When a 1 mM. solution of the drug was passed through the rat intestine at a rate of 1.5 ml. per minute, 24 per cent of the sulfanilamide was absorbed while no absorption was observed for the guanidine derivative. Sulfanilamide is un-ionized at body pH and is somewhat soluble in chloroform, while sulfaguanidine is highly ionized and insoluble in chloroform.

Although quantitative absorption data is unavailable for the other sulfonamides, it is of interest to compare the structures of the compounds known to be clinically effective by mouth and those absorbed poorly, if at all. Figure 109 gives the structures of four compounds and the percentage un-ionized at pH 7, calculated from the published pK_a values.[1] The pK_a of the substituted amide group of sulfathiazole and sulfamerazine is about 7 so that at this pH equal amounts of ionized and un-ionized molecules would be present. Sulfaguanidine and succinylsulfathiazole exist mainly as ions at pH 7. On theoretical grounds, one might expect absorption of the weak acid, succinylsulfathiazole, by the stomach, but apparently it is poorly absorbed as it does not produce an appreciable

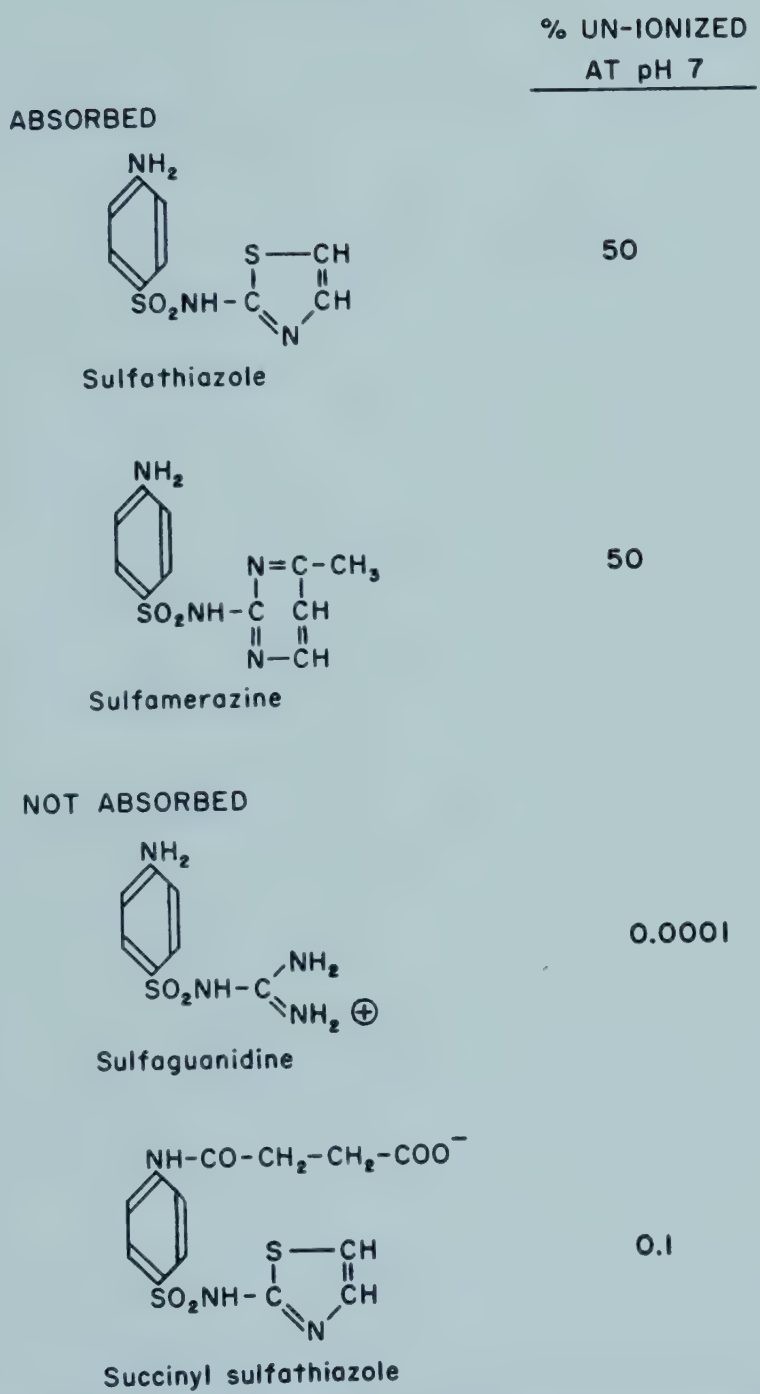


Figure 109. Correlation between chemical structure of sulfonamides and their intestinal absorption.

blood level in man. Since succinylsulfathiazole has about 1 part out of 1,000 un-ionized at pH 7, and this probably is somewhat lipid soluble, one might predict that absorption would be greater than the guanidine derivative but less than sulfathiazole. The author was unable to find experimental data relating to this specific question.

ANTIBIOTICS

As in the case of the sulfonamides, there is remarkably little quantitative information on the intestinal absorption of antibiotic drugs. A comparison of the chemical structures and lipid solubilities of a few compounds is shown in Figure 110.

There are no quantitative data on penicillin absorption from the intestine although it is known that oral administration requires about five times the parenteral dose for similar clinical effectiveness. Some of the drug is apparently destroyed in the stomach by low pH, and poor absorption may be another factor. In spite of the low pK_a of penicillin (2.5) large amounts would be un-ionized in the stomach (pH 1 to 3) and the high lipid solubility[34] would allow rapid absorption. Schanker et al.[29] have shown that compounds with a pK_a of between 2 and 3 are absorbed by rat intestine at an appreciable rate, although more slowly than weaker acids. Active transport of penicillin, such as occurs in the renal tubules, probably does not occur in the intestine since phenol red[29] and diodrast,[15] which are transported in the kidney by the same system as penicillin, are not actively transported in the gut.

Erythromycin and carbomycin are weak bases, of which appreciable fractions exist in the undissociated form in the ileum where the normal pH ranges from 7 to 8. These compounds are well absorbed as would be anticipated from their high degree of lipid solubility in the undissociated form. Streptomycin and bacitracin, on the other hand, which are both highly charged compounds, are poorly absorbed by the intestine.

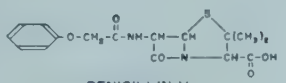
Tetracycline is an example of a drug which is clinically effective when administered orally in spite of its limited absorption from the intestine.[6, 14] In man, for example, a dose of 250 mg. every 6 hours is incompletely absorbed and some appears in the feces.[6] The total capacity for absorption in man, therefore, must be only a few hundred milligrams per day.

QUATERNARY AMMONIUM COMPOUNDS

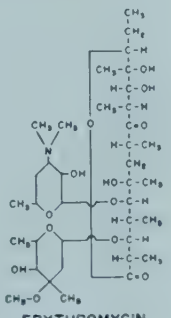
A consideration of this group of compounds is important because a number of valuable drugs fall into this category. It has been pointed out earlier in this chapter that bases with pK_a values above about 10 are not absorbed to an appreciable extent. Tetraethylammonium ion, for example, is a strong base and is not appreciably absorbed in the rat under the conditions of the experiments of Schanker et al.[29]

There appear to be striking exceptions to the generalization given above. It has been shown that choline is absorbed by the dog in considerable amounts (as much as 200 mg. hr. kg. body weight).[28] A possible explanation of the anomalous behavior of choline is that the intestine

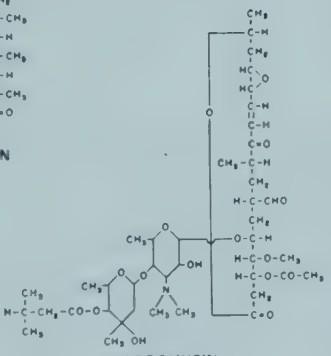
ABSORBED



PENICILLIN V
(MW = 350)



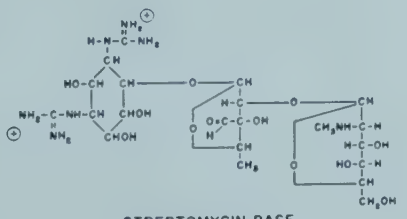
ERYTHROMYCIN
(MW = 734)



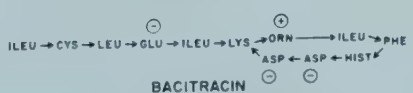
CARBOMYCIN
(MW = 842)

SOLUBILITY (mg./ml.)		
WATER	ETHER	CHLORO-FORM
0.9	12	>20
2.1	>20	>20
0.3	14	>20

NOT ABSORBED



STREPTOMYCIN BASE
(MW = 582)



BACITRACIN
(MW ABOUT 1460)

SOLUBILITY (mg./ml.)		
WATER	ETHER	CHLORO-FORM
>20	0.01	0
>20	0.07	0

Figure 110. Correlation between chemical structure of antibiotics and their intestinal absorption.

possesses some type of special carrier-mediated transport mechanism. The possibility of active transport against a concentration gradient was tested with the intestine of the golden hamster. Choline added to both sides of the gut wall at a concentration of 5 mM, did not move from mucosal to serosal side against a concentration gradient.[35] Many other experimental conditions with different animals must be tested before a final statement can be made.

Another quaternary ammonium compound which is absorbed to a significant extent is hexamethonium. Man is apparently able to absorb from the intestine 5 to 10 per cent of an oral dose of this compound. Levine et al.[16-19] have noted significant absorption of a wide variety of similar compounds. No satisfactory explanation for the absorption of these strong bases is available at present, although some mechanism other than simple diffusion is suspected.

MISCELLANEOUS COMPOUNDS

Nonelectrolytes

The water-soluble sugars and polyhydric alcohols are probably absorbed either by diffusion through water-filled channels or by active transport (see Chapter 4). In the rat, the pore size is apparently large enough to admit tetratols and pentatols but not hexitols.[8] There are many compounds of small molecular weight, such as ethanol, which probably are absorbed by this pore route.

Some drugs, such as trinitroglycerol, are nonelectrolytes with such lipid solubility that they can penetrate almost any cell of the G.I. tract. In this case the oral mucosa is an efficient organ for absorption. Compounds such as CO_2 are both small and lipid soluble, and therefore pass freely across cell membranes. See a review on absorption by the stomach by Karel.[13]

Large molecules

Many large molecules, such as proteins, are absorbed by the normal adult small intestine, but only in minute amounts under ordinary conditions. The mechanism of absorption of these substances is entirely unknown although a variety of hypotheses have been proposed (see Chapter 10).

Heparin, which is a polymer of glucosamine, hexuronic acid, and sulfate,[2] is normally not absorbed to a measurable extent by the small intestine. Loomis[20] has shown that small but measurable absorption occurs if the pH of the lumen is reduced to 1 or 2. The author's view was that sufficient amounts of un-ionized species are present to enter the epithelial cell. Whether or not the explanation is correct, the experimental findings appear to be clear cut.

RECIRCULATION OF DRUGS THROUGH THE G.I. TRACT

When a drug is secreted in one part of the body and resorbed in another a cyclic process results. The best-known example of this phenomenon is, of course, the secretion of substances in the bile and resorption in the small intestine. Another example might be the secretion of iodide by the salivary gland and resorption by the stomach or intestine. Zawoiski et al.[37] have studied another such example. Mecamylamine, a basic drug, is "secreted" into the stomach and resorbed by the small intestine. Undoubtedly this occurs in the cases of many, if not all, weak bases in the blood. The secretion of weak acids in the alkaline secretions of the pancreas and the bile also occurs and many of these compounds are probably resorbed in the small intestine. The recirculation of compounds through the G.I. tract is certainly a common event and is probably much more common than is currently believed.

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